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VOLUME LXX NUMBER 3

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

March, 1942

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PUBLISHED MONTHLY
THE JOHNS HOPKINS PRESS, Baltimore, Maryland
Made in United States of America

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BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

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Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.85 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS
BALTIMORE, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

RAT-BITE FEVER

A Review of the American Cases with Reevaluation of Etiology; Report of Cases

THOMAS McP. BROWN and JOHN C. NUNEMAKER

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Submitted for publication January 12, 1942

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INTRODUCTION

Few diseases adequately recorded in the medical literature are at present in a more confused state with regard to etiology than rat-bite fever. The reason for this confusion has been the fact that two apparently different microörganisms, *Spirillum minus*, and *Streptobacillus moniliformis*, have been considered individually responsible for the same clinical picture in man. The authoritative work of Bayne-Jones (4) and others (119, 123, 95, 41, 89), and the standard text books of medicine have emphasized in the past the causative rôle of *Spirillum minus* in rat-bite fever. At the present time, except for those particularly interested in this field, the general belief still holds that *Spirillum minus* is *the* cause of rat-bite fever.

There has been increasing recent evidence that *Streptobacillus moniliformis* may hold a place of even greater importance than *Spirillum minus* as an etiological agent in this disease. In the entire medical literature the total number of cases of rat-bite fever from which *Streptobacillus moniliformis* has been recovered is still small. It is of interest, however, that in recent years there has been a decided upswing in the number of these cases reported. A reasonable explanation would be the increased general knowledge of the rôle of *Streptobacillus moniliformis* in rat-bite fever, and therefore, greater attention to the specific culture methods which are required to isolate this microörganism.

The writers have accumulated further evidence to support the etiological rôle of *Streptobacillus moniliformis* in this disease. However, it is believed that a proper evaluation of this subject should include the salient information concerning the position of both of these agents in rat-bite fever, and that at the present time one must accept the assumption that both *Spirillum minus* and *Streptobacillus moniliformis* are capable of producing a similar clinical picture in man.

If the foregoing point of view is correct, it becomes apparent that the entire subject of rat-bite fever needs to be completely reevaluated. The writers have, therefore, chosen to discuss this subject from the angle of both *Spirillum minus* and *Streptobacillus moniliformis* in accordance with the outline presented in the table of contents.

GENERAL HISTORICAL RESUMÉ

Illness following rat-bite has probably been known for many centuries, but the first recorded evidence that this fact was appreciated by physicians was supplied by Blumer (see Francis (36)) who found that Professor Eli Ives of Yale lectured on the dangers of rat-bite and the symptoms of the disease, in 1831. Wilcox (158) in 1839, was the first to report the disease in a medical journal using the title, "Violent Symptoms from the Bite of a Rat".

Although Millot-Carpentier (94) in 1884, was probably the first to report the disease in Europe, it remained for Miyake (95) in 1900, to arouse the interest of European physicians in the disease as an entity. He used the name *Rattenbisskrankheit* and disclosed that both Japanese physicians and laity had long been familiar with the disease. His was the first detailed study which defined the entire clinical picture, and included etiological investigations and 11 case histories. Except for the occasional cultivation of *Staphylococcus aureus* from the bite wound, his etiological studies failed.

In 1908, Ogata (108) used the word *Rattenbisskrankheit* in his title, but referred to *sodoku* as the Japanese term for the disease. He was also able to demonstrate in local wound, gland, and blood of his patients, structures which he took to represent protozoons belonging to the Neosporidia, and to which he gave the name *Sporozoa muris*. The microörganisms he demonstrated stained with Giemsa after being fixed in slightly acid-bichlorid alcohol. He was also able to infect rabbits and guinea pigs with a lymph gland excised during life from one of the patients and to demonstrate the same microörganism in the animals. In a later article (1913–14) Ogata (109) added a description of filamentous forms and changed the classification of his microörganism to an Aspergillus.

von Ofenheim (153) in 1909 cited a case of septicemia following rat-bite in a patient who showed erythema, relapsing fever, and arthritis. He isolated *Micrococcus tetragenus* from the erythematous areas and also from the blood stream. Also in 1909, Shikami (Futaki et al., 1917) (41) believed the disease to be caused by a Telosporidium.

In 1910, Horder (54) reported three cases, using the term "Rat-bite Fever", and Middleton (93) described one case under the same title.

From this latter patient, fresh blood films showed tiny rod-like microörganisms in the red cells, and one of several culture tubes (anaerobic gelatin) showed growth of a nonencapsulated diplococcus which was unfamiliar to the investigators and not studied further.

In 1911, Proescher (119) wrote an excellent review of the subject, but clung to the term "Rat-bite Disease" as used by Miyake and Ogata. His article is of interest because of his practically literal translation of much of the work of the two Japanese. Of further interest is his report of a case from which the local wound was excised. Half was studied histologically, and half was implanted into four rats and one guinea pig. The guinea pig became ill in three weeks with swollen glands and skin lesions. Although *cultures were sterile*, histological examination of both the excised human wound and the guinea pig gland showed great numbers of tiny bacilli, straight and slightly curved, with round and slightly pointed ends, and staining either irregularly or in bipolar fashion. It was significant that he followed Ogata's method and used as a fixative bichlorid-alcohol (2-1) and stained with dilute Giemsa solution. "The bacilli could not be stained in sections fixed in formalin, either with Giemsa, Gram or other usual stains." Pictures of the microörganism in his article should be compared with Plate III, Fig. 16 of the present paper. He named the microörganism *Bacillus septico muris*.

Hata (48), in 1912, first suggested and demonstrated the efficacy of arsphenamine. This had occurred to him because of the superficial clinical similarity between sodoku and syphilis.

Schottmüller (129), in 1914, published his classic report of the isolation of a streptothrix in a case of *Bisskrankheit*. From his first patient, bitten by a rat, a Gram-positive (blau-tingiert) streptothrix was isolated and named *Streptothrix muris ratti*. From his second patient, bitten by an African ground squirrel (*Teraxerus cepapi*), a Gram-negative Streptothrix was isolated and named *Streptothrix teraxeri cepapi*.

Until 1916 then, although the disease was clinically well recognized and had been adequately described in the world literature, etiological studies had added nothing but confusion to the picture. No one had confirmed previous work, and several different agents had been incriminated. The year 1916 is significant, because in

consecutive numbers of the *Journal of Experimental Medicine*, volume 23, two articles appeared under similar titles but by different investigators. In January of that year, Blake (7) reported as "The etiology of rat-bite fever" the *Streptothrix muris ratti* of Schottmüller. In February, Futaki, Takaki, Taniguchi and Osumi (40) reported as "The cause of rat-bite fever" a new microörganism, a spirochaete which they had not yet named.

In 1918, Douglas, Colebrook and Fleming (27) reported the isolation of *Streptococcus pyogenes* from a patient with rat-bite fever, but were unable to demonstrate spirochaetes.

RAT-BITE FEVER DUE TO SPIRILLUM MINUS—SODOKU

In 1917, Futaki *et al.* (41) further elaborated on their studies of the etiological agent in sodoku and named it *Spirochaeta morsus muris*. Whereas their original article contained a drawing showing spirochaetes of varying size and shape, the second article indicated the microörganism to be somewhat longer and thicker than *Treponema pallidum*, with 1.5 to 6 curves, and with terminal flagella. The drawings and photographs showed microörganisms quite unlike those in the original article.

They believed the spirochaete to be present in the blood of 3 per cent of house rats in Japan, although in 43 rats, they found the microorganism only once. They asserted that the microörganism had been cultivated on Shimamine's medium, but Bayne-Jones (4) testifies that the Japanese workers later withdrew this claim. Ishiwara, Ohtawara and Tamura (55) demonstrated that guinea pigs could be infected fatally by the bite of a wild rat and that rats and mice bore the infection without ill effect. They were able to find the microörganism in the blood only once in many examinations of normal rats and mice.

Those early articles stimulated other Japanese workers to produce a series of papers during the next few years, all confirmatory in nature. In 1917, Kitagawa and Mukoyama (61), using animal inoculation for diagnosis, attempted to divide the spirochaetes into two groups on a basis of minor differences in size and shape—Type A was recovered from guinea pigs and Type B from rats. Kusama, Kobayashi and Kosai (73) extended comparative morphological and immunological studies to human and animal strains, indicating their identity.

Mooser (98) demonstrated the microörganism in a wild rat in Mexico, and later in the human disease (99).

Joekes (58) asserted that the spirochaete could be grown on culture media, but at no time was he able to free his culture of a motile coliform microörganism also present. Since then, there has been uniform failure by competent investigators who have attempted its cultivation. In spite of this, the microörganism has come to be accepted by the majority of physicians throughout the world as the cause of rat-bite fever.

The term *Spirochaeta morsus muris* became untenable because of its trinomial character, and Robertson (122) believed that because of its rigid body and terminal flagella the microörganism should be included under the spirilla. He, therefore, suggested as more acceptable the name *Spirillum minus*, Carter, 1887, thus identifying it as the first spiral microörganism to be described from a rodent.

Bayne-Jones (4) commented on the lack of epidemics of this disease in the presence of an apparently world-wide spread. It is true that there have been no naturally occurring epidemics of sodoku. On the other hand, there have been recorded several artificial "epidemics," owing to the use of "induced" *Spirillum minus* infection in the treatment of general paresis. There have been three such "controlled" epidemics, dating from 1926, when the practice was initiated by Solomon *et al.* (136). Two more series were reported by Hershfield *et al.* (52), in 1929, and by Teitelbaum (142), in 1930. In each series, patients with central nervous system syphilis were inoculated with blood from guinea pigs or rats presumably infected only with *Spirillum minus*. This large group of cases has been alluded to by only a few writers, and although an excellent source from which to study the clinical characteristics of sodoku, it has been entirely neglected. Following the final analysis of Neymann and Koenig in 1931 (105), the method was dropped as being definitely less effective than malaria or hyperthermia in the treatment of general paresis. Induced *Spirillum minus* infection will be discussed later under the term *Sodoku inoculata*.

A. Clinical Picture of Sodoku

According to present day description, the disease is usually contracted from the bite of a rat, but the literature embraces a host

of other animal sources. The initial wound is supposed to heal without signs of local inflammation. Roughly 7 to 21 days later, the disease begins with the development of an angry purplish indurated area at the site of the bite, usually without suppuration, but occasionally with gangrene. Lymphangitis and regional, or rarely generalized, lymphadenitis develops and the patient has fever, occasionally with chills. After a variable initial period of several days, the fever subsides, and frequently the degree of local wound inflammation regresses somewhat. Following several afebrile days, a second bout of fever occurs, the temperature rises to 105° or 106°F. at times, and again subsides in one or two days. This relapsing type of fever is "typical," and is usually accompanied by increased signs of inflammation in the wound and glands and the appearance of a rash.

Rash is an almost invariable accompaniment of the first or later exacerbations. It is usually dark and purplish, and has been described variously: usually as maculo-papular, but also may be large, small, urticarial, petechial, pustular, hemorrhagic, morbilliform, etc. O'Leary (110), writing with special emphasis on the dermatologic aspects of the disease, says, "The exanthematic phase of rat-bite fever may be graded as mild or severe, and classified under the individual groups of roseola, indurated erythematous plaques, urticarial manifestations, lesions of the mucous membranes, and alopecia." It may involve even the soles of the feet, or may consist of only one or two spots on the trunk or at the site of the bite. It frequently begins around the region of the bite and spreads occasionally to involve the entire body, especially the locality of joints. Severe muscle pains and occasional joint pains are usually a feature of some stage of the disease.

Moderate anemia is usually present, and leucocytosis varies usually from 10,000 to 20,000. The occurrence of false positive Wassermann reactions has been known for years, and the phrase "positive in about 50% of the cases" can probably be attributed to Blum and Clément (10). In Bayne-Jones' 1931 review, only three out of 18 cases with recorded Wassermann reports gave positive reactions, and in two of those syphilis was likely. In his own case, as reported, there was little to support a diagnosis of syphilis. Some cases are supposed to subside spontaneously, and others to last for months and rarely,

years. Response to treatment with one of the anti-syphilitic arsenicals is dramatic and complete.

Few writers have improved on Miyake's (95) original description of the disease, and most have knowingly or unknowingly quoted portions of his paper. Certain omissions in the modern writings, however, seem to demand a reconsideration of the early descriptions by men who were entirely dependent upon "clinical" methods for diagnosis. Proescher's (119), "translation" includes Misoguchi's division of the disease into four types: "First, local symptoms predominating, as inflammation, gangrene combined with edema and bluish-red exanthema surrounding the seat of the bite or the entire body; second, characterized by intermittent fever attacks, combined with miliary papules and, later, vesicular exanthema on entire body; third, beginning with joint pain and followed by the symptoms of the second form; fourth, predominating sensorimotor disturbances of the central nervous system combined with exanthema. The patients exhibit symptoms of paresthesia, creeping disturbance of sensibility, and disturbance of motility of the legs with decreased reflex."

Regarding the fever, Miyake divides the disease into three forms: "First, febrile form with exanthema; second, afebrile form with predominating nervous symptoms; third, an abortive form."

The incubation period in Miyake's 11 cases varied from two to 24 days. He was able to observe the entire course of the disease in five cases, and in each patient he noted an acute urticaria in the phase preceding general improvement. It was associated with itching severe enough to prevent sleep, but was looked upon as a favorable prognostic sign. Miyake found very little involvement of the circulatory or the respiratory systems, "with the exception of a few cases with a slight bronchitis, expectoration of a slimy sputum, and, in severe attacks, marked dyspnea." Regarding the alimentary canal, he stated, "There is a complete loss of appetite during the fever attack, but it is relatively normal in the interval. Sometimes there is a feeling of fullness or dull pain in the gastric region, nausea, and vomiting. In a chronic course of the disease, profuse diarrhea or obstipation is observed. The gastric region is sometimes painful on pressure." Pernicious vomiting has been reported, but was not observed by Miyake. Liver and spleen were not supposed to be

enlarged; nephritis was reported in a few cases. Miyake further noted that the older physicians were accustomed to look upon edema as an unfavorable prognostic sign, whether resulting from nephritis or cachexia.

Apparently, those early physicians who had seen a large number of cases were impressed by the striking nervous system involvement which occasionally occurred. There were marked motor or sensory disturbances, clouding of the sensorium, headache, tinnitus, vertigo, anopsia, delirium, stupor, or deep coma. Miyake laid great stress upon the constancy of the occurrence of muscular pains which became worse during exacerbations of fever, and were occasionally so intense as to prevent sleep. He postulated three cardinal points, in addition to the bite, in making a diagnosis: "First, the intermittent fever; second, the bluish-red exanthema; third, the muscular pain. One or more of these symptoms is present in every case, or in doubtful cases, the anamnesis is considered for the diagnosis."

B. Laboratory Diagnosis of Sodoku

Since the reports of the various Japanese workers from 1916 to 1919, it has become an accepted practice to attempt to substantiate the clinical diagnosis by demonstration of the causative microörganism. The ease with which this has been done has apparently born no relation to the effort expended. Dark-field examination of local wound or regional gland has only occasionally disclosed spiral microörganisms. By far the most frequently used method has been animal inoculation with material from the patient.

Animal inoculation and subsequent repeated examination are time-consuming but essential. The writers did not pursue this procedure in their first case. The same error has been made by numerous previous investigators. The tendency has been to neglect animal inoculation once a spirillum has been observed by dark-field examination of the fluid from the local wound or regional gland. White mice and guinea pigs are the animals of choice, and at least two guinea pigs and four mice should be inoculated. The guinea pigs are best inoculated in the abdomen, subcutaneously, in the hope that inguinal glands will develop, and also intracutaneously into the skin of the genitalia. By this means, it has been shown that a chancre may de-

velop from which the spirilla may be seen by dark-field examination (reference should be made to the second article by Futaki *et al.* (41), and to Francis (36) for excellent illustrations of the *Spirillum minus*). Inguinal nodes, when present, should be punctured and likewise examined. Later, abdominal fluid or blood becomes infected, and the animals usually succumb in typical fashion in one to several months. Some workers have been able to demonstrate a latent infection in guinea pigs which became evident only after blind passage of the blood or organs to other animals. White mice, when infected, harbor the spirilla without evidence of illness, and it is a mistake to expect them to sicken and die. They may show no signs of the infection even when there is a heavy infestation of their blood or peritoneal fluid by spirilla. Examination of the mice for at least one month is essential before discarding them as negative.

Das Gupta (19) showed that the spirilla in infected mouse blood might fluctuate markedly in number from day to day and even temporarily disappear, while in the same mice, spirilla in the peritoneal fluid might not diminish to an appreciable extent. For that reason, he suggested routine examination of peritoneal fluid in preference to tail blood.

Inoculation with patient's blood alone may not always be sufficient. If an accessible gland draining the region of the bite wound can be excised, one portion should be ground, emulsified, and used for inoculation, and another portion cultured. Histological examination of such glands is not usually helpful. In Bayne-Jones' (3) case, although guinea pig inoculation with blood failed, a portion of the patient's involved skin was excised, ground, and injected into a guinea pig with resultant *Spirillum minus* infection.

One great and oft-repeated objection to this method of diagnosis is the natural occurrence of spiral microörganisms in laboratory mice, rats, and guinea pigs.

In 1887, Van Dyke Carter (17) demonstrated a spiral microörganism in the blood of one of 117 Indian wild rats, and designated it *Spirillum minor*. Borrel (11) first described spirochaetes in mouse tumors. Wenyon, (157) found a spiral microörganism in the blood of a gray mouse and named it *Spirochaeta muris*. Both he and Borrel were aware of the occurrence of spiral microörganisms in the alimentary tract of mice. Wenyon then injected mice subcutaneously with

intestinal mucus from both normal and "infected" mice, but was unable to cause an infection with the bowel microörganism. He concluded that the *Sp. muris* infection of the blood had not come from the intestine. Breinl and Kinghorn (12) described a similar microörganism in a mouse infected with trypanosomes, and named it *Spirochaeta laverani*.

Hoffman (53) had described spiral microörganisms in human ulcerated tumors of cervix, face and abdomen. Löwenthal (83) also described spiral microörganisms in ulcerated human tumors, dog tumors, and feces. Friedenthal (39) published photographs of similar microörganisms from non-ulcerated human tumors. Löwenthal named his microörganism *Sp. microgyrata*. Gaylord (43) described a spiral microörganism in mouse tumors, and Calkins (16) gave it the name *Sp. microgyrata* (Löwenthal) *var. Gaylordi*.

In 1907, MacNeal (86) found a spiral microörganism in the blood of one of 39 wild rats in West Virginia, and suggested the name *Spirochaeta muris, var. Virginiana*. He felt that it was morphologically identical with the microörganism of Borrel and of Wenyon, and was the first to demonstrate terminal flagella. Kusama *et al.* (73) pointed out that MacNeal's demonstration of flagella was after advent of dark-field illumination, and inferred that failure of the earlier investigators to demonstrate flagella was from lack of this type of apparatus.

After the accurate descriptions of the microörganisms by Futaki *et al.* (41), numerous other investigators reported searching for similar microörganisms. Kusama *et al.* (73) report an incidence of two in 24 (8.3 per cent) Japanese wild rats, and quote others as 21 of 186 (11.2 per cent), Ishiawara, 5 of 84 (5.9 per cent), Matsuzaki, and 8 of 58 (13.8 percent), Tsuneoka. Parmanand (114) found 13 of 300 (4.3 per cent) Bombay rats infected with spirilla. Seeman (Lanford and Lawson (75)) found no spirochaetes in "3 or 4 dozen" New Orleans rats. Joekes (58) found the microörganism in one quarter of the wild rats examined in London. Bayne-Jones (3) found none in 25 rats examined from Rochester, New York. Leadingham (77) noted none in 73 wild rats in the vicinity of Atlanta, Georgia. Robertson (123) observed spontaneous infection in stock laboratory mice on four occasions in six years. Francis (38) found 65 out of 150 (43 per cent) of one batch of white mice received from a "jobber" to be infected but three other lots from dealers who maintained their own breeders were free of infection. Knowles, Das Gupta and Sen (71) found that 15 out of 31 (48 per cent) white mice, all of one batch, harbored *Spirillum minus* in their blood. Also of interest are Knowles' and Das Gupta's (70) studies on rat infections: Using stained blood films, they found three of more than 100 Calcutta rats to be infected, but when they shifted to dark ground illumination, they found five out of 23 (21.7 per cent) infected. Allbritten *et al.* (1) found 12 laboratory rats negative for *Sp. minus*.

Das Gupta (20) examined the blood of 20 young guinea pigs and noted that they were negative for spirilla, but later found that of 42 guinea pigs infected with Leptospira, four harbored *Sp. minus*,

and they were seen in fairly large numbers in the peritoneal fluid but not in the blood. This may possibly vitiate the negative results obtained by blood examination alone prior to inoculation, and makes it desirable to examine peritoneal fluids of all animals at least twice before inoculating with human blood.

In the hope of controlling any hypothetical non-specific foreign protein effect of human material in activating a latent undiscovered infection in mice used for diagnostic inoculation, the writers have determined on the following course:

Four mice should be inoculated and four more from the same batch saved in a separate cage. If the first group becomes infected after inoculation with material from the patient, the second group should be inoculated with similar material obtained from the same patient after clinical cure is established. This procedure was suggested by the work of Das Gupta.

C. Sodoku in the United States

Following the lead of Bayne-Jones' (4) excellent review of the subject in 1931, we have chosen to limit ourselves to a critical review of all the cases in the United States after 1916 from which an etiological agent has been obtained (Table I). Table II includes four additional cases which were omitted from Bayne-Jones Table I and adds to his list the 40 cases reported in the United States for the 10 years from 1931 through 1940, making a grand total of 125 cases.

Because of inferences in recent years that possibly the spirillum may not actually be involved at all, but may only have been picked up from the particular laboratory animals used for diagnosis, it seems worthwhile to survey the evidence upon which we tentatively accept *Spirillum minus* as being responsible for at least one type of the disease in the United States.

The spirilla have been allegedly seen by direct dark-field examination of the blood or serum of a few cases. Dembo *et al.* (22) reported positive a dark-field examination on lymph from the local wound, but their description of the morphology of the microörganism is sketchy. Ward (155) reported that darkfield examinations on fluids from a cervical node of a patient showed "a spirochaete of 3 spirals and many motile cocci". Also dark-field examination of this patient's blood showed "many micrococci". Zahorsky (160), using dark-field illumi-

nation with India ink, reported that serum expressed by puncture of the original wound showed "*sp. morsus-muris* in large numbers". Steiner (Francis, 1932 (36)) reported having found the microorganism in smears of a patient's blood and photographed it, but Steiner's 1930 article (137) contained no photographs, and only stated that "some spirochaetes were found", without mention of material or method of examination. Therefore, we have not included this case among the five proved by direct examination. Marshall (87) reported that a "small amount of saline was injected into the area around the bite, and smear showed numerous dimly-staining microörganisms resembling spirochaetes, presumably morsus muris". Gilkey and Dennie (44) in a report of one case merely stated that spirochaetes were found in fluid from the wound by dark-field examination. Such descriptions as the foregoing must be classed as inadequate, particularly since in none of these cases was the presence of *Spirillum minus* confirmed by animal inoculation. In our opinion, the etiology cannot be properly established on the basis of direct stain or dark-field examination alone.

The case of Foncannon (35), where *Sp. morsus muris* was said to have been isolated in blood culture, the writers prefer not to accept as of proven etiology.

Diagnosis by animal inoculation is next to be considered. Here, many of the reported cases give very meager information. Altogether there have been 17 such reported cases in the United States from that of Shattuck and Theiler (130) in 1924 to that of Gilkey and Dennie (44) in 1939. Only a few of the reported cases can be considered to have been well-studied, and only Reitzel, *et al.* (120), and Bloch and Baldock (8) mentioned negative dark-field examinations of mice prior to the inoculation with human blood. In the case of Mock and Morrow (96), from the inoculated guinea pigs only one microörganism was found and it was reported to be a spirillum; however, the case has been included in this series. In only 9 of the 17 cases was the blood cultured. In the other 8 cases, either there was no mention of blood cultures or it was stated that none were made.

D. Clinical Analysis of Sodoku

The acceptable American cases of rat-bite fever due to the spirillum will be reviewed in an attempt to see whether the etiologically "proven"

TABLE I

Rat-Bite Fever Cases of "Proved" Etiology in the United States Since 1916

CASE	AUTHOR	YEAR	STATE	SEX	RACE	AGE	SOURCE	ORGANISM	DIAGNOSTIC METHOD	STS	W.B.C.	TREATMENT	REMARKS
1	Blake (7)	1916	Mass.	F	W	67	Rat-bite	*Streptothrix*	Culture of blood	Negative	22,100	Non-specific	Died
2	Tileston (143)	1916	Conn.	F	—	46	Rat-bite	*Streptothrix*	Direct—stain of blood	Negative	24,000	Arsphenamine	Recovered
3	Litterer (82)	1917	Tenn.	M	—	14	Rat-bite	*Streptothrix*	Culture of blood	—	18,700	Autogenous vaccine	Recovered
4	Litterer	1917	Tenn.	M	—	5	Rat-bite	*Streptothrix*	Culture of blood	—	21,000	Autogenous vaccine	Recovered
5	Dick and Tunnicliff (23)	1918	Ill.	M	—	10	Weasel-bite	*Streptothrix putrii*	Culture of blood	Pos. (syphilis)	19,000	Neoarsphenamine	Recovered
6	Tunnicliff and Mayer (148)	1918	Ill.	F	C	13	Rat-bite	*Streptothrix*	Culture of blood	Negative	32,000	Non-specific	Died
7	Shattuck and Theiler (130)	1924	Mass.	F	W	½	Rat-bite	*Spirillum minus*	Blood into mice and g. pigs	Negative	12,600	Cod liver oil and mercury	Recovered
8	Dembo et al. (22)	1925	Ohio	F	—	1/12	Rat-bite	*Spirillum minus*	Direct—D.-field of lymph	Negative	20,400	Sulpharsphenamine	Recovered
9	Lanford and Lawson (76)	1923	La.	—		Child	Rat-bite	*Spirillum minus*	Lymph into mice	—	—	? Arsphenamine	Recovered
10	Dodd (26)	1926	Md.	F	W	10	Rat-bite	*S. moniliformis*	Culture of blood	Negative	14,400	Non-specific	Recovered
11	Ward (155)	1926	N. Car.	M	W	3.5	Rat-bite	*Spirillum minus*	Direct—D.-field of lymph	Positive	6,200	Sulpharsphenamine	Recovered
12	Zahorsky (160)	1926	Mo.	—	—	—	Rat-bite	*Spirillum minus*	Direct—D.-field of lymph	—	—	Sulpharsphenamine	Recovered
13	Bayne-Jones (3)	1927	N. Y.	M	W	48	Rat-bite	*Spirillum minus*	Skin biopsy into one g. pig	Positive	—	Arsphenamine	Recovered
14	Leadingham (77)	1928	Ga	M	W	20	Rat-bite	*Spirillum minus*	Blood into mice	—	18,200	Arsphenamine	Recovered
15	Leadingham	1928	Ga.	M	W	11	Rat-bite	*Spirillum minus*	Blood into mice	Positive	8,400	Arsphenamine	Recovered
16	Francis (36)	1932		M	—	6	Rat-bite (?)	*Spirillum minus*	Gland lymph into white rat	—	—	Arsphenamine	Recovered
17	Anderson and Spector (2)	1932	Ill.	M	C	4	Rat-bites	*Sporothrix*	Culture of blood and lymph	—	—	Quinine and potassium iodide	Recovered

18	Mock and Morrow (96)	1932	Ill.	M	W	43	Cat-bite	*Spirillum minus* (1)	Blood into guinea pig	Positive	22,000	KI, Hg and neoarsphenamine	Recovered
19	Hazard and Goodkind (49)	1932	Mass.	M	—	58	Trauma	*S. moniliformis*	Culture of blood	—	10,500	Non-specific	Recovered
20	Ripley and Van Sant (121)	1934	Ill.	M	—	26	Dog contact	*Spirillum minus*	Blood into mice, g. pigs, and dogs	Positive	33,200	Arsenical #16 (Lilly)	Recovered
21	Ripley and Van Sant	1934	Ill.	M	—	22	Dog contact	*Spirillum minus*	Blood into mice, g. pigs, and dogs	Positive	23,600	Neoarsphenamine	Recovered
22	Scharles and Seastone (128)	1934	Mass.	M	—	Adult	Rat-bite	*S. moniliformis*	Culture of joint fluid	—	18,400	Neoarsphenamine	Recovered
23	Koerber (72)	1935	Calif.	M	—	12	Rat-bite	*Spirillum minus*	Gland into rat. Blood into g. pig	—	14,900	Arsphenamine	Recovered
24	Reitzel et al. (120)	1936	Calif.	M	—	14	Mouse-bite	*Spirillum minus*	Gland and blood into mice	Negative	7,900	Neoarsphenamine	Recovered
25	Wooley (159)	1936	Mass.	M	W	6½	Rat-bite	*Spirillum minus*	Gland and blood into g. pigs	Positive	25,000	Neoarsphenamine	Recovered
26	Marshall (87)	1936	Ky.	F	W	2	Rat-bite	*Spirillum minus*	Direct—stain of lymph (wound)	Positive	30,500	Stovarsol	Recovered
27	Bloch and Baldock (8)	1937	Ky.	F	W	3	Rat-bite	*Spirillum minus*	Blood into mice	Positive	8,850	Sulpharsphenamine	Recovered
28	Walker (154)	1937	Va.	F	W	16	Trauma	*Spirillum minus*	Blood into g. pig	Negative	24,600	Neoarsphenamine	Recovered
29	Walker (154)	1937	Va.	F	W	50	Cat-scratch	*Spirillum minus*	Blood into g. pig	Negative	15,600	Neoarsphenamine	Recovered
30	Leadingham (78)	1938	Ga.	M	W	7	Rat-bite	*Spirillum minus*	Blood into mice	Negative	19,100	Neoarsphenamine	Recovered
31	Dawson and Hobby (21)	1939	N. Y.	M	—	Adult	Rat-bite	*S. moniliformis*	Skin and blood culture	Positive	—	PABS (sulfanilamide)	Recovered
32	Dawson and Hobby	1939	N. Y.	M	—	Adult	Rat-bite	*S. moniliformis*	Culture of blood	Positive	—	PABS and arsphenamine	Recovered
33	Gilkey and Dennie (44)	1939	Mo.	F	W	5	Rat-bite	*Spirillum minus*	Direct—D.-field on lymph (wound)	Positive	—	Arsphenamine	Recovered
34	Gilkey and Dennie	1939	Mo.	M	—	3	Rat-bite	*Spirillum minus*	Lymph gland into mouse	Negative	16,700	Neoarsphenamine	Recovered
35	Farrell et al. (31)	1939	N. Y.	F	W	40	Rat-bite	*S. moniliformis*	Culture of blood (7)	Negative	11,450	Aspirin, codeine, PABS, thymol	Recovered
36	Allbritten et al. (1)	1940	Penn.	M	—	21	Rat-bite	*S. moniliformis*	Culture of blood (3)	Negative	32,000	KI, blood, neoarsphenamine	Recovered

TABLE II

A Continuation of Bayne-Jones' Table I (4) with Additions and 40 New Cases through 1940

CASE	AUTHOR	YEAR	STATE	SEX	AGE	SOURCE	ORGANISM*	DIAGNOSTIC METHOD	SITE	TREATMENT	REMARKS
38	Dick and Tunnicliff (23)	1917	Ill.			(Same case reported by Brennemann) *Streptothrix putorii* isolated (probably syphilitic)					
82	Litterer (82)	1916	Tenn.	M	5	Rat-bite	*Streptothrix*	Blood cultures	Negative	Autogenous vaccine	
83	Dodd (26)	1926	Md.	F	10	Rat-bite	*? H. multiformis*	Blood cultures	?	—	Retrospect. diag.
84	Mulherin (100)	1928	Ga.		Child	Rat-bite	—	—	—	Neoarsphenamine	(These two cases
85	Steiner (137)	1930	Conn.	M	33	Rat-bite	*Spirillum minus*	Blood cultures, direct examination	Negative	Salvarsan	omitted by Bayne-Jones)
	The above cases finish Bayne-Jones' compilation through 1930										
	40 additional cases from 1931 through 1940										
86	Orr (111)	1931	Ky.	M	5	Rat-bite	—	—	Positive	Neoarsphenamine	
87	Francis (36)	1932	Va.	M	6	Rat-bite	*? Spirillum minus*	Animals	—	Arsphenamine	
88	Caldwell and Templeton (15)	1932	Wis.	F	28	Rat-bite	—	Cultures and animals	Positive	Arsenical #158 and neoarsph.	Poor response to Bi.
89	Jenkinson and Jordan (57)	1932	Iowa	M	56	Mouse-bite	—	Animals	Negative	Neoarsphenamine	
90	Anderson and Spector (2)	1932	Ill.	M	4	Rat-bite	*Sporothrix*	Cultures and animals	—	Quinine and KI	Agglutinins 1/2560
91	Mock and Morrow (96)	1932	Ill.	M	43	Cat-bite	*Spirillum minus*	Cultures and animals	Positive	KI, Hg, and neoarsphenamine	One spirillum found
92	Hazard and Goodkind (49)	1932	Mass.	M	58	Trauma	*H. multiformis*	Cultures	—	Orthopedic	"Spont." case (arthritis)
93	Bloom (9)	1933	La.	M	2½	Rat-bite	—	Animals	—	Sulpharsphenamine	
94	van der Bogert (151)	1933	N. Y.		2½	Rat-bite	—	Animals	Negative	Sulpharsphenamine	Complic. by dysentery
95	James (56)	1933	Tenn.	F	30	Rat-bite	—	Cultures	Negative	Neoarsphenamine	Resid. symptoms 5 mos.
96	Ripley and Van Sant (121)	1934	Ill.	M	26	Dog contact	*Spirillum minus*	Cultures and animals	Positive	Arsenical #16	Transm. to dog
97	Ripley and Van Sant	1934	Ill.	M	2	Dog contact	*Spirillum minus*	Cultures and animals	Positive	Neoarsphenamine	Transm. to dog and human
98	Chapman and Chapman (18)	1934	Texas	F	40	Rat-bite	—	—	Negative	Neoarsphenamine	

99	Chapman and Chapman	1934	Texas	M		Mouse-bite	—	—	—	Neoarsphenamine	
100	Edelman and Haber (29)	1934	Ohio	M	5	Rat-bite	—	Animals	Positive	Sulpharsphenamine	Parents non-syphilitic
101	Edelman and Haber	1934	Ohio	F	8	Rat-bite	—	—	Positive	—	#100 and 101 siblings
102	Edelman and Haber	1934	Ohio	F		Rat-bite	—	Animals	Positive	Sulpharsphenamine	
103	Scharles and Seastone (128)	1934	Mass.	M		Rat-bite	*H. multiformis*	Cultures and animals	—	Neoarsphenamine	Agglutinins 1/512 (arthritis)
104	Koerber (72)	1935	Calif.	M	12	Rat-bite	*Spirillum minus*	Cultures and animals	—	Arsphenamine	
105	Reitzel et al. (120)	1936	Calif.	M	14	Mouse-bite	*Spirillum minus*	Cultures and animals	Negative	Neoarsphenamine	
									Positive	Neoarsphenamine	Syphilis ruled out
106	Wooley (159)	1936	Mass.	M	6½	Rat-bite	*Spirillum minus*	Animals	Positive	Bi and Hg	
107	Marshall (87)	1936	Ky.	M	2	Rat-bite	—	—			
108	Marshall	1936	Ky.	M	2	Rat-bite	*Spirochaetes*	Direct stain	Positive	Stovarsol	
109	Marshall	1936	Ky.	M	8	Rat-bite	—	—	Negative	Bi and stovarsol	Arthritis and sore throat
110	Bloch and Baldock (8)	1937	Ky.	F	3	Rat-bite	*Spirillum minus*	Animals	Positive	Sulpharsphenamine	
111	Garvin (42)	1937	Ohio	M	7	Rat-bite	—	—	—	Sulpharsphenamine	
112	Walker (154)	1937	Va.	F	16	Trauma	*Spirillum minus*	Cultures and animals	Negative	Neoarsphenamine	
113	Walker	1937	Va.	F	50	Cat-scratch	*Spirillum minus*	Cultures and animals	Negative	Neoarsphenamine	
114	Leadingham (78)	1938	Ga.	M	79	Rat-bite	—	Cultures and animals	Negative	Neoarsphenamine	
115	Leadingham	1938	Ga.	M	7	Rat-bite	*Spirillum minus*	Cultures and animals	Negative	Neoarsphenamine	
116	Fisher (33)	1938	W. Va.	M	3	Rat-bite	—	—	—	Neoarsphenamine	
117	Dawson and Hobby (21)	1939	N. Y.	M		Rat-bite	*S. moniliformis*	Cultures	Positive	PABS and arsphenamine	Polyarthritis
118	Dawson and Hobby	1939	N. Y.	M		Rat-bite	*S. moniliformis*	Cultures	Positive	PABS	Polyarthritis
119	Hadley (46)	1939	D. C.	M	42	Rat-bite	—	—	Negative	Neoarsphenamine	
120	Gilkey and Dennie (44)	1939	Mo.	F	5	Rat-bite	—	Animals	Positive	Neoarsphenamine	
121	Gilkey and Dennie	1939	Mo.	F	5	Rat-bite	—	Animals	—	Neoarsphenamine and Bi	
122	Gilkey and Dennie	1939	Mo.	F	5	Rat-bite	*Spirillum minus*	Direct examination	Positive	Arsphenamine	
123	Gilkey and Dennie	1939	Mo.	M	3	Rat-bite	*Spirillum minus*	Animals	Negative	Neoarsphenamine	
124	Farrell et al. (31)	1939	N. Y.	F	40	Rat-bite	*H. multiformis*	Cultures	Negative	Salicyl., PABS, thymol	Arthritis for 7 mos.
125	Allbritten et al. (1)	1940	Penn.	M	21	Rat-bite	*H. multiformis*	Cultures and animals	Negative	KI, blood, and Neoarsph.	Arthritis

* In the above table, it is not meant that the *Spirillum minus* was ever obtained by cultures.

cases conform to the "typical" clinical picture. The five cases diagnosed by direct examination of the patients' fluids have already been mentioned and will not be considered further, since data on all are too scanty to evaluate. There have been only 17 cases in which the spirillum was demonstrated by animal inoculation.

1. *Source of infection.* In only 11 of the 17 was rat-bite definitely the cause. In one, rat-bite was only supposed, one was from mouse-bite, two were from contact with dogs (in only one of which was there trauma), one from cat-bite, one from cat-scratch, and finally Walker's case (154) in which a 16 year old female sustained trauma to her knee while at a carnival without any known animal contact.

2. *Site of wound.* This is usually on the hands or feet, but some patients have been bitten on the face, and Francis' patient presented a wound on the penis.

3. *Incubation period or date of onset.* This is very difficult to determine fairly. In some cases it dates from the first sign of local inflammation, and in others from the first febrile bout after initial subsidence of the wound. The briefest incubation period is in case 8 where symptoms began the day following the bite. The longest incubation period is in Leadingham's case (77) where symptoms first appeared 36 days after the bite. In the majority, however, onset occurred after an interval of 7 to 21 days.

4. *Local inflammation in the wound.* Although this is supposed almost invariably to accompany exacerbations of the fever, the patients of Leadingham, Bloch and Baldock, and Walker showed no local reactions at all. In the case of Mock and Morrow, the local inflammation lasted 10 days. In case 20, it persisted for 2½ months. In cases 23 and 28, there was generalized adenopathy.

5. *Rash.* Skin eruption of some type has been almost always present. Yet it is striking that so many reports contain the word typical without giving any description. A perusal of the "proven" cases reveals that there is actually great variation in occurrence and type of exanthem.

In case 7, the first American case, it was described as *brownish-red* on the patient's trunk and extremities, and occurred 16 days after onset of symptoms. In Case 8, the patient had rash only at the local site and never had a generalized rash at all. In case 23 of Koerber, the patient had no rash at any time. In

case 25 of Wooley, the patient had rash during only one period of fever, which consisted of "only a vague blush on abdomen and chest, neither eruptive nor distinctive." Ripley and Van Sant's patient (20) had severe generalized lesions for one month; these were described as "more than a simple rash." Their second patient (21) had red itchy lesions on back and arms, red tender nodules on the forearms, and diffuse mottled macular eruption on the chest with each bout of fever. The nodules seem to have been quite unlike Moore and Davis' description of painless cutaneous nodules from Sporotricosis following mouse bite (97).

6. *Relapsing character of fever.* This is supposed to be one of the most characteristic features of the disease, yet marked variations appear in the reported "proven" cases.

In case 7 of Shattuck and Theiler, the patient had five bouts in 20 days, reaching 102–104 F. The patient in case 8 had 11 febrile peaks in 23 days—not typical relapsing fever. Leadingham's patient (14), after 35 days of incubation, had the first sustained rise for 9 days, the second for 6 days, and the third for one day, before treatment was started. In case 18, the patient had periodic chills and fever for three months with five relapses during 68 hospital days. The patient in case 20 of Ripley and Van Sant, after seven days of incubation, had intermittent daily fever for 17 days, then regularly relapsing fever every five days for the next three weeks. Their second patient (21) had intermittent fever for 1½ months, and then apparently relapsing fever for 2 weeks. In case 30 of Leadingham, the patient, after a two-week incubation period, suddenly developed chills and fever, with daily recurrences of fever for several weeks. Eighty days later, the febrile rises began to occur at "lengthening intervals." In case 16 of Francis, the patient had seven relapses in 39 days with roughly one day of fever each week. Case 19 of Koerber could be called *typical* of sodoku. Local symptoms in the wound were first noticed three weeks after the bite. Five weeks after the bite the first fever occurred with temperature of 105.8°F. Thereafter, febrile relapses recurred every Monday and Tuesday for four weeks, until treatment was started.

7. *Leucocytosis.* The presence of an elevated white blood cell count is directly related to the presence of fever. In many cases, the count was normal during remission. Of 16 cases with adequate data, the white blood cell count was above 30,000 in one case; above 20,000 in five cases; above 10,000 in six cases; and below 10,000 in four cases.

8. *Serologic test for syphilis(STS).* Following Bayne-Jones' (4) survey, considerable doubt has been thrown on the false positive STS. For that reason, several investigators have thoroughly studied

this problem. Of the total number of 22 cases of spirillary rat-bite fever, the STS was reported in 17 and unknown in five. Of the 17 reported, 10 (59 per cent) were positive and seven negative. However, of the 17 reported, four cases were diagnosed *Spirillum minus* infection only by direct dark-field examination without animal inoculation and the ratio there was three positive to one negative. If these four cases were eliminated, there would remain seven positive and six negative tests in the cases diagnosed by animal inoculation. So on this basis, it may be said that false positive serologic tests for syphilis occur in spirillary rat-bite fever in 50 to 60 per cent of the cases.

In case 25 of Wooley, a 6½ year old male, the flocculation test was originally negative, but later became positive on four occasions, negative once, and doubtful twice. The Wassermann test was negative on two occasions. Syphilis was ruled out by negative family and personal history and by negative serologic examination of the parents. It is obvious that in some cases the term "Wassermann" test has been taken by the authors as meaning any STS, and for that reason it is well to note that actual complement fixation tests may not have been made.

There may be positive complement fixation and negative flocculation tests in one individual at the same time, as shown in the case of Bloch and Baldock (27) where the Wassermann test was 2+ while the Kahn test was negative. Two corrections should be made in Bayne-Jones' Table I (4). His case #66 of Leadingham, a male, age 11, had a positive Wassermann test, no evidence of syphilis, and the parents' bloods were negative. Also, in his case #59 of Ward, the patient had a 4+ Wassermann test with cholesterinized antigen, which was confirmed by two separate serologists. Here also, the parents' Wassermann tests were negative.

In this connection it is pertinent to cite certain experimental data regarding this point of false positive STS in *Spirillum minus* infections.

Ripley and Van Sant reported two cases in medical students who had been working with dogs in physiological experiments. One student injured his finger with a ligature, but the other suffered no known trauma. Both students contracted "rat-bite" fever with atypical clinical courses, and both developed strongly positive Kahn reactions in the course of the disease. In the first (case 20), Wassermann and Kahn tests were both negative 23 days after the bite, although symptoms had begun 20 days previously. The Kahn reaction became 3+, 88 days after the bite. In each case blood was inoculated into mice, guinea pigs, and a dog. Spirochaetes were isolated from the mice and were found in the eyes of

the guinea pigs. The first dog (case 20) sickened and later died, and its blood injected into mice caused *Sp. minus* infection. In the second case (case 21), the inoculated dog recovered after having received treatment, and finally developed strongly positive Wassermann and Kahn reactions three months later.

Das Gupta (1938), in investigating this problem further, inoculated two human volunteers who denied syphilis and had negative Wassermann reactions. He used a strain originally isolated from a human case and maintained in mice. After both patients had contracted a "generalized infection, as shown by the isolation of the specific parasite from the blood by animal inoculation (guinea pigs), their blood was tested again, and gave absolutely negative reactions." Unfortunately, no statement is made of repeated serologic examinations at later dates. Of the four guinea pigs involved in the experiment, before infection, three had negative and one had doubtful Wassermann reactions. After a well-established infection had occurred in all of the inoculated pigs, the Wassermann tests on the blood of all four animals were definitely positive. This is contrary to the experience of Bayne-Jones (1927), who states that he has "never found a positive Wassermann reaction to occur with the blood-serum of experimentally infected guinea pigs."

This also seems to provide further evidence for accepting *Spirillum minus* as the responsible agent in sodoku. Unfortunately, Das Gupta does not mention control blood cultures in any of his experiments.

9. *Response to arsenicals.* This is another problem difficult of evaluation. Many cases were treated late in the course of the disease when natural subsidence might have been expected; however, there are enough records on hand to leave little doubt that arsenicals may dramatically arrest the fever at least.

Of 18 cases suitable for analysis, neoarsphenamine was used in seven, arsphenamine in five, sulpharsphenamine in three, arsenical number 16 (Lilly) in one, potassium iodide, mercury, and neoarsphenamine in one, and cod liver oil and mercury in one case. Following the first injection of arsenic, there was an immediate exacerbation of fever not unlike a Herxheimer reaction in a fair proportion of cases—13, 20, 21, 24, and 29, or in 28 per cent of the 18 cases. In case 7, a child with rickets was treated only with cod liver oil and mercury because the parent refused to allow "needle treatments." Four months later, the child still had a palpable liver and spleen and to the authors looked like a patient with a chronic infection. Case 18 of Mock and Morrow was given potassium iodide and mercury rubs for an indefinite period, but up to three months after the bite, continued to have paroxysms every 10 to 14 days with fever to 102–104°F. and subsidence in 48 hours. Three injections of neoarsphenamine were then given at three-day intervals, and there were no more febrile relapses; however, four months later, the patient still had soreness of arm, thigh, and leg muscles.

It is also apparent that one injection of arsenic frequently failed to effect complete cures in many cases. Only two cures (cases 11 and 15) were noted after a single injection. Relapses after inadequate treatment have been frequent.

In case 15 of Leadingham, the patient was given three injections of arsphenamine at five and six day intervals, and the temperature became normal two days after the first treatment; however, 30 days after completion of the treatment, the patient was readmitted to the hospital with adenitis, fever, and persistently positive Wassermann reaction. Five more injections of arsphenamine were administered in the next month, and cure presumably resulted. In case 8 of Dembo *et al.*, the patient was given three injections of sulpharsphenamine at two-day intervals, and the authors claimed rapid improvement in the next week in spite of several paroxysms of fever. The patient in case 11 of Ward was given one injection of sulpharsphenamine, but four weeks later had another relapse and was given two more treatments with "marked improvement." In case 23 of Koerber, which is so typical clinically, the patient was given three treatments with arsphenamine at four-day intervals. According to the authors, there were no relapses, but one month after treatment the patient had one or two attacks of malaise and slight fever, and also developed a red rash in the right axilla at the site of a biopsy. In case 24 of Reitzel, *et al.*, the patient was given three injections of neoarsphenamine at two-day intervals with a marked febrile rise after the first treatment. The patient was well until 18 days after the last injection, when he had another relapse with temperature of 103°F. and required a second course of treatment.

From these data then, it would seem that arsenic brings about a dramatic response, but that if treatment is not maintained for more than three injections, relapses may occur.

10. *Complications in sodoku.* In the United States through 1940, death has never been reported in a case of rat-bite fever from which *Spirillum minus* has been isolated.

During the course of sodoku, and occasionally following cure, there occur complications which have seldom been emphasized.

The patient in case 8 was considered for a time to have meningitis because of extreme hyperesthesia, bulging fontanelle, and neck rigidity. During convalescence a paroxysmal cough developed which was diagnosed as pertussis. In case 11 of Ward, the patient had paralysis of the palate and left leg after the first injection of sulpharsphenamine, but this did not recur following subsequent treatments. Case 6 of Mock and Morrow was very interesting. Two days after cat-bite, the patient had a painful swollen hand that was treated by incision

and drainage. He was discharged from the hospital two weeks later, but with a productive cough, and pains and soreness through chest, abdomen and legs. He was readmitted three months later after having had periodic chills and fever, continued cough, shortness of breath, pains as before, and weight loss of thirty pounds. There was pitting edema, and the patient was thought to have "chest pathology on the right." The response of fever to arsenic treatment was good, but as previously mentioned, pain persisted for four months. Six months later, the patient was well. The two patients of Ripley and Van Sant both developed epididymitis, and in addition one had acute bilateral episcleritis, while the other had slight conjunctivitis and palpable spleen. The patients in cases 23 and 24 both had palpable spleens. In case 27, the patient had a palpable spleen, and definite joint pains. This was the case of trauma to the right knee and no history of animal contact. Onset was characterized by local pain in the right knee, seven days after the injury. With the second relapse, there was involvement of the right elbow and left ankle and associated fever and rash. In case 28 the patient had tenderness over the spleen. The patient in case 30 had severe aching pains over the entire body.

E. Sodoku Inoculata

Additional proof of the etiologic rôle of the spirillum seems to have been established by inoculation of infected animal blood into humans with the reproduction of the "typical" disease. Unfortunately, no mention has been made of cultures of the infecting animal blood, although they must have been made in some instances. Suffice to say there has been no report of any disease other than sodoku resulting from such inoculations. The practice was instituted in the United States by Solomon *et al.* (136), in 1926, for the treatment of general paresis; this was the first of two "controlled" epidemics. Twelve paretics were inoculated either intradermally or intravenously with 0.1 to 1.0 cc. of citrated heart blood of infected guinea pigs or rats, using the strain of spirillum of Shattuck and Theiler first isolated in this country. The typical disease resulted in all, and active spirochaeticidal antibodies were demonstrated in the blood of all the patients during and after the disease, by the use of two different methods. Bayne-Jones (4) (1927) was able to cause the typical disease in a paretic by inoculating with the blood of a guinea pig which had been infected from a patient with rat-bite fever. Hershfield *et al.* (52) (1929) achieved identical results in 72 patients using 0.2 cc. subcutaneously of fresh whole blood from infected guinea pigs. Teitelbaum (142) (1930) was also successful in 15 cases by

giving 2.0 cc. intramuscularly of citrated guinea pig heart blood (1.0 cc. of heart blood, 1.0 cc. of 1 per cent sodium citrate). In 1930, McCluskie (88), in England, tested three humans with a naturally occurring mouse strain of spirilla, and was unable either to induce typical sodoku in the patient or to transmit the disease back to the mice from the patients. In 1934, Ripley and Van Sant (121), using blood of a mouse infected from their first patient, were able to cause the typical disease in a patient with dementia paralytica, and a guinea pig was in turn fatally infected with this paretic's blood. Knowles, Das Gupta and Sen (71) working in India, inoculated a normal male Hindu volunteer with blood from a mouse which was one of a group known to be spontaneously infected with *Spirillum minus*. The typical disease resulted, and the patient's blood was infectious for a young guinea pig which had been found negative for spirilla by two previous examinations of blood. In 1938, Das Gupta inoculated two human volunteers with a strain of spirillum originally obtained from a human infection and maintained by mouse passage. Here again, typical sodoku resulted and the infectious agent was transmitted in both cases to guinea pigs.

It has been considered important to summarize the symptomatology of the patients in whom the infection was induced by injection of *Spirillum minus* in order to formulate a comparison between the induced and naturally occurring infections.

In the entire English literature, we have noted 104 cases of artificially induced infection by inoculation with *Spirillum minus*. Our interest has been chiefly directed to the clinical observations recorded in the largest single group reported, the 72 cases of Hershfield, *et al.* (52).

The infection developed after inoculation by a variety of routes—intracutaneously, intramuscularly, subcutaneously, and intravenously.

Either guinea pig or rat blood was generally used in amounts of 0.1 to 2.0 cc. (Das Gupta in India, used mouse blood). Hershfield was unable to transmit the disease from man to man.

A feature of the induced disease was the early appearance of a local lesion: within three to five days after intracutaneous infection. After subcutaneous inoculation, there was an initial walnut-sized induration which disappeared after a few days, and reappeared later.

The degree of inflammation in the local lesion, except after intravenous inoculation, usually became extremely severe. In some cases the lesion was four to six inches in diameter with a central area of necrosis which finally resulted in a punched-out ulcer one to four inches across, and usually oval in shape. This chancre-like sore persisted throughout the disease, so that local exacerbation with each bout of fever was difficult to determine.

Lymphangitis and adenitis were regular features. In Hershfield's 72 cases, regional adenitis was present in 75 per cent and distant adenitis in 15 per cent. A few patients even showed epitrochlear gland enlargement.

Onset of fever occurred 6 to 21 days after inoculation in the entire series, and in 10 to 15 days when the spirilla were administered by the subcutaneous or intramuscular routes. By the intracutaneous route, fever developed in 6 to 8 days, and by the intravenous route, in 7 to 21 days (Solomon). The febrile rises varied from 102° to 106°F., and occurred at three to four-day intervals. Each cycle of temperature elevation lasted from one to five days.

Rash usually began in 14 days, but of great interest was its presence in only 25 per cent of Hershfield's and 20 per cent of Teitelbaum's cases.

There was a moderate anemia in practically all of Hershfield's cases.

Spontaneous cures occurred in 12.5 per cent of Hershfield's patients where the disease was allowed to run 32 days before treatment was instituted. In Teitelbaum's series, the paroxysms were allowed to run for 6 weeks, and because of this longer period, spontaneous cures were noted in 55.5 per cent of the 15 cases.

Active spirochaetocidal antibodies were demonstrated in Solomon's cases.

The Herxheimer type of reaction in response to arsenicals was commented upon by both Solomon and Teitelbaum. Good therapeutic results followed arsphenamine, neoarsphenamine, and sulpharsphenamine. Hershfield's 72 cases were apparently all treated with neoarsphenamine, and in 50 per cent of his cases, one injection of 0.3 gm. sufficed for cure. In 1931, Neymann and Koenig (105), in a follow-up report on this series of cases wrote, "We have become

convinced that it is much more difficult to arrest this disease than is generally accepted. One injection of 0.3 gm. of neoarsphenamine does not necessarily terminate the fever or prevent sequellae; often five or six injections are necessary."

Complications form one of the most interesting features in the analysis of these artificially induced cases of sodoku, and they were reported in detail only by Hershfield *et al.* One patient died from myocarditis and two from pneumonia, although it was said by the authors that only two of the deaths could be attributed to the induced infection. One patient lost control of "all organic reflexes", two had disorders of the heart and dyspnea, two had delirium and convulsions, and eight (9 per cent) had severe muscle involvement with swelling, redness and severe pain. Most interesting of all, and a feature not commented upon by previous writers, was the occurrence of *arthritis* in 18 (25 per cent) of the 72 cases. There was swelling, redness and pain in the joints, the elbow and ankle were most commonly involved, and in many instances the arthritis was migratory in character.

In the 1931 report of Neymann and Koenig, 50 of the original 72 cases were summarized, and the mortality was 5 out of 50, or 10 per cent. In their table, those cases are listed as deaths due to the spirillary infection, without any further qualification. The high motrality in their series might be attributed to the use of a more virulent strain. This is in contrast to McCluskie's (1930) report wherein his mouse strain was avirulent for man.

HISTORY OF THE STREPTOBACILLUS MONILIFORMIS

As mentioned in the beginning, Schottmüller (129) in 1914 was the first to describe adequately the streptothrix which he cultured from the blood of a patient suffering from rat-bite fever. His name for the microörganism was *Streptothrix muris ratti*, and for a similar microorganism obtained by culture from a patient bitten by a squirrel *Streptothrix teraxeri cepapi*. In 1916, Blake (7) reported the isolation of a very similar microörganism in a fatal case of rat-bite fever in the United States. Tileston (143) next reported a case which was well-studied from the standpoint of adequate cultural procedure as well as inoculation, but no microörganism was obtained. Blood smears, on the other hand, revealed filamentous forms which suggested a streptothrix.

In the ensuing two years, four more cases were reported in the American literature. Litterer (82) obtained a microörganism from each of two cases by blood culture, and named them *Streptothrix longus* and *Streptothrix brevis* because of morphological differences only. Dick and Tunnicliff (23) obtained a new microörganism which they named *Streptothrix putorii* because the patient had been bitten by a weasel. Tunnicliff and Mayer (148) in 1918 also reported a fatal case from which a similar streptothrix was cultured. Litterer's excellent description of this microörganism was the first to include the term *streptobacillus* in reference to its morphology.

In 1925, Levaditi, Nicolau and Poincloux (80) reported a case of "acute erythema multiforme", unassociated with rat-bite, from which a microörganism was cultivated differing from Schottmüller's streptothrix only in being Gram-negative. Being unaware of the isolation of that microörganism 11 years previously, they named it *Streptobacillus moniliformis* because of its distinctive morphology. In 1937, Lemierre *et al.* (79) reported the recovery of the same microörganism from the blood of a patient with fever from rat-bite.

In 1926, Place, Sutton and Willner (117) reported an epidemic, at Haverhill, Massachusetts, of a disease which they named *Erythema arthriticum epidemicum*. A peculiar microörganism was cultured from both the blood and joint fluid of the patients. Parker and Hudson (113) who studied this organism in detail, placed it in the family *Mycobacteriaceae*, but since it differed from *Actinomyces bovis* and the *Actinobacillus* of Lignieres and Spitz, they created a new genus and named it *Haverhillia multiformis*. Here again, the authors were unaware of both Schottmüller's streptothrix and Levaditi's streptobacillus, which were the same microörganisms as the newly named *Haverhillia multiformis*.

In the seven years following the report of *Haverhill fever*, two more cases were reported under that title. That of Dodd (26) in 1926 was due to rat-bite, but was not so mentioned in the title; while that of Hazard and Goodkind (49) in 1932 was associated apparently with trauma, but not with animal contact. Eight years after the report of Place *et al.*, Scharles and Seastone (128), in 1934, reported a case of "Haverhill Fever following Rat-Bite" and due to *Haverhillia multiformis*. So, after a 16-year interval in American literature, rat-bite "disease" was again shown to be due to a microörganism which could

be cultured from the blood of patients without recourse to animal inoculation.

Small wonder that there should have been so much confusion regarding the existence of this microörganism when it appeared at first under three different names, had been described in three different languages, had been isolated in three different countries, had been obtained in patients suffering from three "different" conditions and had been transmitted by three different mechanisms. It is now agreed by all that the microörganism was similar if not identical in all of the three instances.

Animal Reservoirs of Streptobacillus moniliformis and Reference to the Confusion in Nomenclature

In 1916, Tunnicliff (147) described a streptothrix in 56 of 60 white rats with bronchopneumonia. She was able to culture it from 20 rats, and observed its presence most frequently in rats sick from lesions in which other bacteria were present. Lung lesions were produced in young rats by intraperitoneal inoculations. Out of 28 wild rats, one was found to be infected, and 24 normal laboratory rats were uninfected. She called attention to the fact that the microörganism, stained by Levaditi's method, closely resembled the spirochaetes pictured in the first article by Futaki *et al.* (40). Growth was obtained on human blood agar and ascitic goat blood agar, and she was aware of the resemblance of this streptothrix to those obtained from cases of rat-bite fever.

In 1918, Theobald Smith (132–134) described a microörganism obtained from calves with pneumonia. It grew in the condensation water of serum-enriched media, and appeared to have "clubs." Because of this similarity to the actinomyces, he named it *Bacillus actinoides*, n. sp.

In 1922, Jones (59) recovered a microörganism which he noted was similar to that of Smith, from pneumonic lungs of 11 albino rats. He did not re-name it.

In 1923, Bergey's Manual (5) listed Smith's microörganism as *Actinomyces actinoides*.

In 1929, Topley and Wilson (144) changed the name to *Actinobacillus actinoides*.

In 1930, Nelson (102) isolated a similar microörganism from the middle ears of albino rats. He made 80 cultures on one or both ears from 50 adult rats (12–22 mos.), and obtained the microörganism 35 times. He named it *Bacillus actinoides, var. muris* (103).

In 1932, Levaditi, Selbie and Schoen (81) reported the occurrence of "spontaneous infectious rheumatism" in mice due to a strain of *Streptobacillus moniliformis* identical to a strain which they had previously recovered from a human.

In 1933, Strangeways (138) next showed that *Streptobacillus moniliformis* was apparently a normal inhabitant of the nasopharynx in 50 per cent of laboratory

rats. She also found it in four wild rats, but not in the nasopharynx of 16 healthy laboratory mice.

In 1933, Mackie, Van Rooyen and Gilroy (85) reported an epizoötic disease of mice, with eye involvement, gland and joint swelling, and paralysis of the hind legs. Van Rooyen (1936) (152) studied the microörganism obtained and suggested naming it *Haverhillia multiformis*, although he classified it under the tribe *Hemophileae*. He commented on the microörganism described by Tyzzer (1917) (150) as *B. piliformis* (N. Sp.) which was specifically pathogenic for Japanese waltzing mice. Only pathological and morphological comparisons could be made because of lack of cultural studies by Tyzzer. Tyzzer in turn thought his microörganism was closely related to the one obtained by Flexner (1896) (34) from a rabbit and termed *Bacillus* (Leptothrix?) *pyogenes filiformis* (N. Spec.).

In 1936, Topley and Wilson, (145) fully aware of the confusion resulting from this multiplicity of names, suggested the name *Actinomyces muris* for the microorganism described by Schottmüller, Levaditi, *et al.*, and Parker and Hudson; however, they retained the term *Actinobacillus actinoides* for the microörganism described by Smith.

In 1937, Dienes and Edsall (25) called attention to the resemblance between the streptobacillus and the microörganism of Theobald Smith.

Also in 1937, Klieneberger and Steabben (68), having worked intensively with *Streptobacillus moniliformis*, noted its similarity to Smith's *B. actinoides*. They further commented upon the lung condition which obtains so frequently in rats, and which had been known in their laboratory since 1911. They alluded to Meyer's résumé (1928) (91) and further showed that the terms *bronchiectasis*, *bronchopneumonia*, *pneumonia*, *abscess*, and *caseation* all described a similar picture varying apparently with the age of the animal, stage of the disease, and presence of various superimposed bacterial infections. In 17 out of 19 cases, they obtained a microörganism resembling the L_1 microörganism which Klieneberger asserts is a symbiont of *Streptobacillus moniliformis* (1935) (66). In a few severe cases of lung diseases, both microörganisms were found.

In 1940, they (69) further showed that the lung lesions were associated with the presence of a pleuropneumonia-like microörganism which they designated L_3. They examined 251 laboratory rats, from which the microörganism was recovered in 138. The lung lesions were present in 108 rats. Out of 17 wild rats, one had lung lesions and the microörganism. When the series was divided into age groups, it was found that the incidence of lung lesions increased with age so that 100 per cent of the rats over 12 months of age were affected.

It seems worthy of comment that these lung lesions are very similar to those described by McDermott (89) as *gummatoid* lesions of the "tertiary stage" of *Spirillum minus* infection, and from which he felt that *Spirillum minus* had been demonstrated by sub-inoculation of the contained necrotic material into mice and guinea pigs. Leadingham (78) also described "granulomas" which developed in the lungs of old rats used for passage of *Spirillum minus*.

Bergey's Manual (1939) (6) now lists the microörganism of Smith as *Actino-*

TABLE III

Varied Nomenclature for Streptobacillus Moniliformis

YEAR	AUTHOR	ORGANISM	SOURCE
1914	Schottmüller (129)	*Streptothrix muris ratti*	Blood of a human bitten by a rat
1914	Schottmüller (129)	*Streptothrix terazeri cepapi*	Blood and pus of a human bitten by a squirrel
1916	Tunnicliff (147)	*Streptothrix*	Lungs of rats showing bronchopneumonia
1917	Litterer (82)	*Streptothrix longus* and *brevis*	Blood of humans bitten by rats
1918	Dick and Tunnicliff (23)	*Streptothrix putorii*	Blood of human bitten by a weasel
1918	Tunnicliff and Mayer (148)	*Streptothrix* closely related to *Streptothrix putorii*	Heart blood of a human dying following rat bite
1918–1921	Smith (132–134)	*Bacillus actinoides*	Lungs of calves dying of pneumonia
1922	Jones (59)	Organism resembling *Bacillus actinoides*	Lungs of rats showing pneumonia
1923	Bergey et al. (5)	*Actinomyces actinoides*	Proposed name for Smith's *Bacillus actinoides*
1925	Levaditi et al. (80)	*Streptobacillus moniliformis*	Blood from a spontaneous human infection without rat bite
1926	Parker and Hudson (113)	*Haverhillia multiformis*	Blood of humans following epidemic from use of non-pasteurized milk
1929	Topley and Wilson (144)	*Actinobacillus actinoides*	Proposed name for Smith's *Bacillus actinoides*
1930–1931	Nelson (103)	*Bacillus actinoides* variety *muris*	Diseased middle ears of rats
1932	Levaditi et al. (81)	*Streptobacillus moniliformis*	Blood of mice showing "spontaneous infectious rheumatism"
1933	Strangeways (138)	*Streptobacillus moniliformis*	Nasopharynx of laboratory rats
1933	Mackie et al. (85)	*Streptobacillus moniliformis*	Epizootic disease in laboratory mice, found in blood and organs

TABLE III—*Concluded*

YEAR	AUTHOR	ORGANISM	SOURCE
1934	Scharles and Seastone (128)	*Haverhillia multiformis*	Joint fluid of human bitten by a laboratory rat
1936	Topley and Wilson (145)	*Actinomyces muris*	Proposed name for organisms of Schottmüller, Levaditi, and Parker and Hudson
1939	Bergey et al. (6)	*Actinobacillus actinoides*	Acceptance of Topley and Wilson's proposed name for Smith's *B. actinoides*
1940	Nelson (104)	*Actinobacillus muris*	Proposed name for previous organism isolated from infected ears of rats
1941	Sabin (125)	*Murimyces streptobacillimoniliformis*	Proposed name for the L_1 component of *Streptobacillus moniliformis*
1941	Heilman (51)	*Asterococcus muris*	Proposed name for organism isolated both from blood of human bitten by a rat and from rat middle ears

bacillus actinoides, following the lead of Topley and Wilson; however, there is still no listing for *Streptobacillus moniliformis*.

Allbritten *et al.* (1940) (1) were unable to obtain *Streptobacillus moniliformis* from 12 laboratory rats.

In 1940, Nelson (104) in writing further on the microörganism obtained from the middle ears of rats, suggested the name *Actinobacillus muris* in place of *B. actinoides, var. muris*.

Sabin (125), in writing on the pleuropneumonia group, suggested for the L_1 microörganism the term *Murimyces streptobacilli-moniliformis*.

Finally, Heilman (51) adds to this already confusing list the most recent variation in nomenclature, *Asterococcus muris*.

It should be emphasized that when the name *streptobacillus* has been used, the present writers have not referred to the two microörganisms listed in *Bergey's Manual* (6) as: (1) *Streptobacillus* (Rist & Khoury) listed under Family IX. Lactobacteriaceae Oral-Jensen; and (2) *Bacteroides streptobacilliformis* (Hauduroy *et al.*) under *Streptobacillus gracilis* (Guillemot and Hallé) which was a microorganism of uncertain position isolated many years ago from putrid pleurisies.

The foregoing bacteriological account, summarized in Tables III and IV, stresses the truly chaotic state of the nomenclature regarding *Streptobacillus moniliformis*. The lack of a generally acceptable classification has not only obscured much important work on the subject, but has led to the "discovery" of the streptobacillus more than once.

Also from the resumé of the various animal sources, and from the preceding section on *Spirillum minus*, it is apparent that streptobacillus infection occurs in much higher incidence in rats than does *Spirillum minus* "infection". Furthermore, it appears that the streptobacillus, (or an L-form which will be discussed later), is more closely related to upper and lower respiratory tract disease in the rat than is

TABLE IV

Microörganisms Suggestively Similar to Streptobacillus Moniliformis

YEAR	AUTHOR	ORGANISM	SOURCE
1896	Flexner (34)	*Bacillus pyogenes filiformis*	Uterus and lung of laboratory rabbit dying post-partum
1908	Ogata (108)	*Sporozoa muris* (*Neosporidia*)	Blood and gland of human bitten by a rat
1911	Proescher (119)	*Bacillus septico muris*	Excised wound of human bitten by a rat
1914	Ogata (109)	*Aspergillus*	New classification of the organism previously classed with Sporozoa
1917	Tyzzer (150)	*Bacillus piliformis*	Obtained from livers of Japanese Waltzing mice

Spirillum minus. This is also true in mice where the streptobacillus infection may extend to the joints. Tunnicliff (1916) was impressed with the intimate relation of the streptobacillus to the biting mechanism of rats, which could explain transmission of the infectious agent to human beings when bitten. This is of particular significance in view of the unsuccessful attempts of many workers to demonstrate *Spirillum minus* in the mouths of rats.

Allbritten (1) has suggested that the laboratory rat-bite is likely to result in streptobacillus infection, whereas wild rat-bite is apt to produce *Spirillum minus* infection. The present writers believe this difference is more apparent than real, for the reason that individuals bitten by laboratory rats are likely to be more closely studied and,

thereby, to receive the benefit of the special culture methods necessary for the isolation of the streptobacillus.

In the two years 1939 and 1940, nine cases of rat-bite fever have been reported in the United States. In five of these, alleged etiological agents were isolated: *Spirillum minus* once, and *Streptobacillus moniliformis* four times. It is doubtful whether this recent high incidence of streptobacillus infection compared to that of *Spirillum minus*, could be attributed to a sudden alteration in the distribution of the infecting agents. Rather, it would seem more likely that a greater general knowledge of *Streptobacillus moniliformis*, and of the special technics required for its isolation, have been responsible for this change.

The writers' decision to retain the term *Streptobacillus moniliformis* is based on the fact that most of the significant research on this microorganism exists in the literature under that name. Since there was no question of rat-bite in the Haverhill epidemic, it would appear preferable to limit the term *Haverhill fever* to those spontaneous or epidemic cases (due to *Streptobacillus moniliformis*) in which the source of the infection is obscure, or at least not due to rat-bite. When this microörganism is isolated from a patient bitten by a rat, it is suggested that the term *Rat-Bite Fever due to Streptobacillus moniliformis* be used. If all future reports on rat-bite fever include, whenever possible, the designation "due to ——" appended to the title, much aid will be given toward the ultimate determination of the relative importance of *Streptobacillus moniliformis* and *Spirillum minus*. Since the same microörganism is concerned both in rat-bite fever due to *Streptobacillus moniliformis* and in Haverhill fever, there seems little reason to confuse this already complex subject by the use of the equally valid, but less descriptive term, *Haverhillia multiformis*.

HAVERHILL FEVER (STREPTOBACILLUS ETIOLOGY) UNASSOCIATED WITH RAT BITE

In February 1926, Place, Sutton, and Willner (117) made a preliminary report on the occurrence of a new epidemic disease which they named *Haverhill fever*, because the epidemic occurred in Haverhill, Massachusetts. They also suggested the name *Erythema Arthriticum Epidemicum*. Eighty-six persons were affected, and the source of

the infection was traced to unpasteurized milk or ice cream from one dairy which supplied 95.5 per cent of the patients involved. Parker and Hudson (113) isolated the causative microörganism from the blood of 11 of the patients and from the joint fluid of two patients in the epidemic. Eight years later, Place and Sutton (116) summarized the epidemic in an article which established the disease as a definite clinical entity with the following symptomatology:

Incubation period, where it was definitely known, varied from one to three days.

Onset was abrupt, and began usually with chill, vomiting, or severe headache.

Vomiting was present in 61 per cent, and nausea alone in 3.5 per cent of the cases.

Chills were present in 55 per cent, and generally preceded other symptoms.

Headache, fairly severe, was present in 56 per cent of the cases.

Fever, present in every case examined, varied from "slight" to 105°F.

Rash was recognized in 93 per cent of the cases, and the authors (116) believed that it might have been overlooked in some of the cases, since not all of the histories were satisfactory. The eruption appeared one to eight days following the onset and persisted for from one to 21 days. It was distributed chiefly on the lateral and extensor surfaces of the extremities, and was occasionally most marked around the joints. In some cases the rash was on the face and trunk as well as the extremities. In character, it consisted of "blotchy, irregular, generally rounded, maculo-papular, dull red lesions varying greatly in shape and arrangement, and measuring from 1–4 mm. in diameter". In one case the lesions were slightly larger. The rash was chiefly rubelliform, but occasionally tended toward a morbilliform appearance. The most severe cases had petechial hemorrhages over the feet, lower legs and lower arms. In 20 per cent of the cases, there was a fine desquamation.

Arthritis. "This was the most persistent symptom. Its onset was from the 2nd to 13th day of the disease. . . . There were many remissions and recurrences. In addition to definite involvement of the joints there was generalized pain in the arms and legs with no local evidence in the joints." This was particularly true of infants and

children. "About 16% had generalized pain, of whom three fourths were infants and children." The duration of joint involvement varied from two days to five months; in three patients, all over 44 years of age, symptoms were still present 2½ years later. In children and young adults, the arthritis entirely cleared. At the end of eight weeks, persistent joint involvement was still present in exactly twice as many patients over the age of 21 as in those under that age.

Respiratory involvement. Sore throat was present in 45 per cent of the cases, and appeared between the 1st and 25th day of the disease and lasted from one to 25 days.

Dysphagia was present in only three cases.

Cough was present in 24 per cent of the cases and was associated with the slight sore throats. Three patients had pain and tenderness over the larynx; three had acute bronchitis with cough and sputum; two had bronchopneumonia of one week's duration.

Blood. In 27 per cent of the cases, the blood examination revealed a white blood cell count varying from 5,680 to 19,000. There was nothing unusual in the differential counts. Average red cell count was 4 million and hemoglobin 80 per cent.

Cultures were positive for *Haverhillia multiformis* in 11 of 17 cases, and were obtained at periods varying from the 1st to the 25th day of the disease. Joint fluid cultures were attempted in two cases and the microörganism was recovered in each instance.

Course. In the majority of the cases, the *first stage* consisted of the abrupt onset, with fever which reached its height in 24 hours and generally returned to normal in two to five days. The rash usually appeared at the time the temperature was returning to normal. After the remission of fever and an amelioration of symptoms, the *second stage* set in. This occurred after two to three days of the remission and coincided with the onset of generalized joint involvement. During this latter period, the rash frequently increased, and the fever recurred and remained elevated for a few days to weeks, depending upon the severity of the arthritis. "The majority of the fevers, however, were septic, the temperature gradually reaching normal in a week."

Diagnostic methods.

1. Blood or joint-fluid cultures.
2. Specific agglutination of *Haverhillia multiformis* (*Streptobacillus*

moniliformis). Serum from the patients produced agglutinins for a polyvalent antigen in dilutions of 1/50 to 1/100, while normal controls had titres no higher than 1/20. Unfortunately, continued agglutination studies of the patients after recovery was not possible.

3. Skin tests: these were made from four to five months after the infection and were found to be positive (1–3 cm. reaction) in 15 out of 18 cases, while 10 controls were negative.

Treatment. No form of therapy was found to have a specific curative effect. Salicylates, hypnotics and narcotics were freely used. "Sodium salicylate, acetylsalicylic acid, and strontium were administered to the point of toxicity without therapeutic relief. Quinine and the iodides also gave no definite result." One patient received a single injection of neoarsphenamine with slight improvement the next day, but there was no change in the arthritis, which persisted for months. Another patient was given intravenous mercurochrome, but there was no apparent effect on the fever or the arthritis. Orthopedic treatment, such as immobilization of the affected joints, was used. Diathermy, and radiation therapy resulted in some symptomatic relief, but these measures had no effect on the course of the arthritis.

The authors also commented on the epidemic occurring in May and June, 1925, at Chester, Pennsylvania, involving an estimated 600 cases. They concluded, "Although the nature of the epidemic was not determined, there was a striking similarity of onset, symptoms, and course and epidemiologic relation to the milk supply, to the disease reported here".

In searching for the source of the infection, in the Haverhill epidemic, it was finally found that one cow from the single involved dairy showed antibodies against the microörganism isolated from the patients, and routine examination of this cow revealed "only a slight superficial lesion of one teat and dripping from another". The owner then disposed of the cow, and further studies ceased. Parker and Hudson failed to infect a cow with the microörganism, using a variety of routes. One procedure, in retrospect, would have been to try to infect the udder. It seems quite possible to us that a dairy barn might harbor rats, and furthermore, that milch cows with large udders and teats would provide tempting bait for the type of rat which so frequently bites sleeping human beings.

It is perhaps worthy of mention that Alice Evans (1918) (30) reported a streptothrix infection of cows' udders and noted only two other similar observations in the literature. The Gram-positive microörganism was isolated from 18 of 21 samples of milk from one herd. It was probably of little or no pathogenic significance and was considered to be a common saprophyte, since a similar microörganism had been found in the air of stables. The manner by which the microörganism infected the udders was unknown. It should also be recalled that *Bacillus actinoides* obtained by Theobald Smith (132–134) came from pneumonic lungs of calves.

Place and Sutton (116) were of the opinion that the Haverhill epidemic picture was entirely different from the usually described picture of Sodoku, and we are in accord with this view. However, certain of the reported sodoku cases possess so many of the features of Haverhill fever that it seems only fair to suppose that many cases of "atypical" sodoku have been reported without consideration of the possibility that the illness may have been caused by *Streptobacillus moniliformis*. The occurrence of arthritis due to the streptobacillus infection is certainly not a feature usually described in sodoku, yet case 27 (*Spirillum minus* infection) previously cited, did have definite arthritis. Furthermore, the 25 per cent incidence of arthritis in the Chicago "induced" sodoku "epidemic" of Hershfield *et al.*, certainly cannot be disregarded.

RAT-BITE FEVER DUE TO STREPTOBACILLUS MONILIFORMIS

Rat-bite fever has been usually described as having typical symptomatology and course. This typical picture has become so closely associated, in the minds of most physicians, with the presence (real or inferred), of *Spirillum minus*, that the tendency has been to regard the streptobacillus rat-bite fever symptomatology as perceptibly different, and to call this latter entity *Haverhill Fever*. We believe from an analysis of the literature and our own cases, that the two disease entities are grossly similar. Occasionally, however, there have been sufficient clinical differences to justify a possible separation of the two conditions.

Recent papers have attempted to draw a definite line between the clinical features of the two types of rat-bite fever. On the other hand, it has even been suggested by Dawson and Hobby (21) that possibly the majority of the cases reported in the past, may actually have been

due to the streptobacillus, and that the spirillum may only have played a minor rôle which would make a separation impossible. An obvious thought is that both agents may be concerned in every case, with one or the other playing a dominant rôle as reflected by differences in symptomatology. Against this possibility is the evidence that there never has been a case reported in the United States from which both agents have been isolated. It must be realized, however, that seldom has a search been made in the same case for both *Streptobacillus moniliformis* and *Spirillum minus*.

The review of Allbritten, Sheely and Jeffers (1) contains data on "five cases of fever following rat-bite from which both Spirillum minus and a microörganism resembling Haverhillia multiformis were said to have been recovered". Further scrutiny of those five cases reveals the following facts:

a. In 1926, Mackie and McDermott (84) reported a case of rat-bite fever in England from which the spirillum was obtained by animal inoculation. Blood cultures revealed a Gram-negative bacillus which was pleomorphic, but which also demonstrated "exceedingly active motility" due to a single terminal flagellum.

b. Later in 1926, Cadbury (14) reported two cases from China, and from one of them the wound discharge "showed the presence of a streptothrix and also spirochaetes, staphylococci and streptococci". No further studies were made.

c. In 1927, Bayne-Jones (3) reported a case with demonstration of the spirillum by animal inoculation. All blood cultures were negative except for one plate "which was obviously contaminated by a hard, chalky white growth of two colonies of a streptothrix commonly found in dust. This microörganism did not resemble any other streptothrix described in connection with this disease and was not pathogenic".

d. In 1928, Knowles and Das Gupta (70) reported 29 cases from India. In one of them, "the blood of a white mouse inoculated with the blood of a patient suffering from rat-bite fever showed not only *Spirillum minus*, but also a streptothrix. On sub-passage of its blood into a second mouse, the *Spirillum minus* infection took, but not the streptothrix one". No cultural studies were made nor was any description of the streptothrix given other than the name.

e. In 1933, Nathan (101) reported a case from Germany from which

spirochaetes were identified by examination of secretion from the local wound. Histologic examination of the wound revealed "cocci, mycelia, and fungus spores" which "corresponded throughout with the forms of Streptothrix muris ratti described by Shottmüller, and Ebert and Hesse (28)". The photomicrograph demonstrating this reveals nothing but uniform round coccus-like bodies. Neither cultures nor further studies were made.

The writers believe that the above evidence is entirely inadequate for inclusion of these cases as proof of the coexistence of *Spirillum minus* and *Streptobacillus moniliformis* in the same individual. In none of the five cases was the streptobacillus actually isolated. The motility of the Gram-negative bacillus rules out the first case (a.); morphology and non-pathogenicity rules out the third (c.). Knowles and Das Gupta's "streptothrix" (d.), although isolated by mouse inculation could not be passed to another mouse (mice are highly susceptible to streptobacillus infection). Also this is the only instance on record where the microörganism (*Streptobacillus moniliformis*) has been thought to exist in mouse blood in recognizable form. The presence of several different microörganisms in the local wounds of the other two cases (b. and c.) makes identification almost impossible, and because of lack of cultural studies, these cases cannot be accepted. We believe, therefore, that in two of the cases the microörganism was definitely not *Streptobacillus moniliformis*; in the other three cases the data are too inadequate and confusing to merit final opinion.

After eliminating the above five cases from consideration, it is still a fact that there has never been a case reported from which both the spirillum and the streptobacillus have been actually isolated and maintained by animal passage and culture, respectively. Should such a case ever occur, it would be important for the investigators to demonstrate antibodies against one or the other (or both) of the microörganisms in an attempt to determine their comparative antigenicities.

As mentioned previously, the available American literature records very few cases completely studied for the presence of both microörganisms. For a number of years, animal inoculations and direct dark-field examinations have been the only diagnostic procedures employed, and reports on many of the carefully studied cases have not contained any references to blood cultures. On the other hand, in the cases

where blood cultures were made, and were positive for the streptobacillus, adequate animal inoculations were rarely carried out. Therefore, not until every case has had both procedures properly performed will it be possible to establish the primary rôle of either one or the of these agents.

A. Symptomatology

The incubation period in *Streptobacillus moniliformis* infection following rat-bite, is generally considered to be much shorter than the usual one to three weeks for the spirillary form of the disease. Ordinarily, with the streptobacillus infection, the patient develops chills and fever one to three days after the bite; there are few signs of local inflammation and no adenitis; rash is present and varies in type and distribution as it does in the spirillary disease; the fever may be intermittent, but is not as regularly relapsing as in sodoku; joint pains and actual arthritis are common; false positive Wassermann reactions are uncommon but do occur. Response to arsenical therapy cannot be properly evaluated until more cases have received this form of treatment.

B. Clinical Analysis of American Cases and Comparisons with Sodoku

Consideration now turns to the 13 cases in the American literature (Table I) from which the streptobacillus has been isolated. In 12 of these, it was cultured directly, and in one the microörganism was only seen in dark-field examinations and in stained blood films. This was case 2 of Tileston. The microörganism showed numerous filaments, and was observed during paroxysms only. It was thought to be very similar to the streptobacillus isolated by Blake. Cultures on Loeffler's medium and ascitic broth were negative, as were inoculations of the patient's blood into brown rats, rabbits, and guinea pigs. This represents one of the three cases in which both culture and animal inoculation were performed with negative results. While it has been listed with the cases of streptobacillus infection, we have not considered it sufficiently valid to include in this report. Complete analysis of the remaining 12 cases also suffers from the fact that in only three of these cases were animal inoculations performed with the thought in mind of searching for the spirillum.

1. *Source of infection.* Of the 12 cases, rat-bite was known in nine, presumed in one (Francis, 1932 (36)). Weasel-bite occurred in one case, and trauma in one case with no history of animal contact. Rodents, specifically rats, have played a greater rôle in this form of the disease than in the spirillary form.

2. *Site of wound.* No essential difference has been noted between the streptobacillus and spirillum diseases.

3. *Incubation period or date of onset.* This was supposed to be much shorter in the streptobacillus than in the spirillary type, although analysis of the cases does not confirm this belief.

The onset was immediate in case 10 of Dodd. In cases 22 and 36, acute symptoms developed in three days. In Blake's case (1) paroxysms of fever appeared on the 2nd to 6th days. In case 6, it was probably 9 days. Then, surprisingly, onset appeared in 14 days in cases 4, 5, and 35; in 14 to 21 days in cases 31 and 32, and in 22 days in case 3. In case 19 the date of onset was indefinite, possibly months after the trauma.

Here, then, is found an unexpected marked similarity to the sodoku cases.

4. *Signs and type of local wound inflammation.* A parallel has been noted for every case of sodoku reported.

The wound on the index finger of Blake's patient lasted for four days with lymphangitis and axillary adenitis. Litterer described it in his two cases (3 and f) as "typical," having in mind the previous description of sodoku. In both cases 5 and 6 the patient had swelling at the site of the wound and regional adenitis at the time of onset. The local lesion in case 10 was described as immediate and persistent (signs of inflammation). In case 19 of Hazard and Goodkind the patient apparently had an osteomyelitis of one toe. In case 22 of Scharles and Seastone, there was local tenderness and lymphangitis three days after the bite, but no local swelling or adenitis. Dawson and Hobby (cases 31 and 32) made no statement. In case 35 of Farrell, *et al.*, there was no local reaction at all. In case 36 of Allbritten, *et al.*, the patient had no local reaction, but did have moderate generalized adenopathy, and during paroxysms, a regional axillary node became swollen and painful.

5. *Rash.* In the streptobacillus type of the disease, rash has been present in every case without exception. Innumerable descriptions of the rash have been given, some of which are indistinguishable from the rashes described in sodoku. A rash of some type has been described more consistently in streptobacillus cases than in sodoku.

Litterer described it in case 3 as purplish, mottled, and erythematous; and in case 4 as a bluish-red exanthem. In case 5, it occurred with each attack, and in case 6 involved the chest and abdomen. In case 10, it occurred on the 4th day, was petechial on the soles, and formed blebs and pustules later. It was described in case 19 as macules and papules, some hemorrhagic, on legs and arms. In case 22, it was called morbilliform on the legs, arms, back and palms. It was purplish and maculo-papular in cases 31 and 32, maculo-papular and petechial only on the extremities in case 35. In case 36 it was maculo-papular on the chest and abdomen, found also on the extremities, palms, and soles and was more noticeable during the febrile periods. Some were petechial and purpuric.

6. *Character of fever.* Some cases had relapsing fever, while others were atypical and more remittent or septic in type. When the fever was relapsing in character, even if not absolutely periodic, it was indistinguishable from that observed in sodoku.

In Blake's case (1) the fever was atypical. The patients in cases 3 and 4 each had at least five paroxysms with three-day persistency of fever and five to six-day remissions. Case 5 had numerous paroxysms at one to six-day intervals. In case 10 the fever was "atypical" and not relapsing. In case 7, the temperature was intermittent. In case 22, it was typically relapsing. In cases 31 and 32, there were recurrent paroxysms of fever at irregular intervals for weeks. In case 35, there was atypical fever for eight weeks, and in case 36, it was atypical with three relapses—the first for three days, the second for nine, and the third for approximately 26 days.

7. *Leucocytosis.* In the 11 cases with suitable data, there were two with leucocyte counts above 30,000, three above 20,000 and six above 10,000. In this respect, a close parallel has existed between the streptobacillus and spirillum cases. The only point of difference has been the finding of occasional leucocyte counts below 10,000 in sodoku. However, the presence or absence of fever at the time these leucocyte counts were made, was not recorded. In cases 2, 4 and 36, there was an eosinophilia of 4 per cent.

8. *Serologic test for syphilis (STS).* In this respect, there was a moderate difference between the two diseases. In case 5 of Dick and Tunnicliff (23), the patient had a positive Wassermann test, and probably had syphilis, since the STS of the mother and a brother were also positive. In the two cases of Dawson and Hobby (21), both patients had positive Kline tests, and one had a positive Wassermann when both antigens were used, while the other was positive only with

the cholesterol antigen. Negative results were recorded in five cases, and no record was made of any STS reports in the five remaining cases. Therefore, three out of the eight tests were positive, or 37.5 per cent of those in which tests were attempted. This is to be contrasted with the 50–60 per cent positive tests in sodoku; however, the streptobacillus series is too small to allow definite conclusion. The significant fact is that a positive STS may occur in both types of illness.

9. *Response to Arsenical and other Forms of Therapy.* The small number of streptobacillus cases treated with arsenicals makes any attempt to compare them with similarly treated sodoku cases quite inconclusive.

In case 2 of Tileston, the diagnosis was based on stained blood smears, and the patient was given Fowler's solution with no apparent effect. When one injection of arsphenamine was given with the sixth relapse, the effect was striking, and there was no fever thereafter. Unfortunately, this case cannot be regarded as proved streptobacillus infection. In cases 3 and 4 of Litterer both patients, were given identical courses of autogenous streptothrix vaccine composed of microörganisms killed by heating at 56°C. for 15 minutes. The initial dose was 50 million microorganisms, and thereafter, at three to seven-day intervals, increasing doses were given for a total of ten doses. Treatment was begun in both cases after the 5th paroxysm. The patient in case 3, after the first dose of vaccine, had a reaction in six hours with generalized pains and joint pain, headache, and fever of 101°F. Each dose thereafter caused muscle pain and slight fever. The identical situation was noted in the second case. Both patients were well in 10 and 6 months respectively. In case 5 of Dick and Tunnicliff, the patient was given a "small dose" of neoarsphenamine with the onset of the third attack. The result was very poor, many subsequent relapses occurring with fever occasionally as high as 101°F. This patient was discharged after 11 weeks, and still had fever. The patients in cases 1, 6, and 10 received no specific treatment, and although patients 1 and 6 died, patient 10 recovered. The only specific therapy in case 19 was surgical amputation of the toe showing osteomyelitis.

In case 22 of Scharles and Seastone the patient was given 0.3 gm. of neoarsphenamine on the 23rd day of the disease, seven days after the last relapse, and "continued to improve slowly. There were no further attacks of rash or fever, and although the ankle remained somewhat tender and swollen for nearly a month, it eventually cleared up entirely." The patients in cases 31 and 32 were both given sulfanilamide with "somewhat equivocal results." One patient received arsphenamine, but convalescence had already begun, so that Dawson and Hobby were unable to judge its efficacy. In case 35 of Farrell *et al.*, the patient was given salicylates without effect. Codeine was thought to have some beneficial effect. Next, the patient was given sulfanilamide for six days (total 12.7 gm.) during the

5th week of the disease without effect. Next, thymol was given, 1.0 gm. daily, during the 10th, 11th, and 12th weeks, and the authors conclude that it "probably had no specific action." Arsenic was not tried. In case 36 of Allbritten *et al.*, the patient, was given potassium iodide, 2–6 gm. daily from the 12th to the 68th day of the disease. After two bouts of fever, a blood transfusion of 500 cc. was given because of hemoglobin of 68 per cent. The temperature then fell to normal, and the patient was discharged. But on the 56th day of the disease, neoarsphenamine was given because of persistent stiffness in the left hip and temperature ranging from 99° to 100°F. Two days after the first injection, the hip symptoms and fever disappeared. Semiweekly treatments were continued to a total of 2.5 gm. neoarsphenamine. One year later, the patient still had an occasional twinge of pain in the left hip and persistently enlarged lymph nodes in the left axilla.

Thus conclusions as to the efficacy of any one method of treatment are hazardous. Sulfanilamide definitely seems to have no beneficial effect. Arsenic cannot be fairly judged because it has not had a fair trial. Case 2 of Tileston cannot be considered, because the method of diagnosis is inconclusive. Only in case 36 does the patient appear to have benefitted materially from arsenic, but he still had a mild joint symptom one year later. In the other streptobacillus cases, when the patients received arsenical therapy, this form of treatment was either administered too late in the disease or after too many other procedures, to be judged fairly.

10. *Complications in rat-bite fever due to the streptobacillus.* In the United States through 1940 the mortality from rat-bite fever, in those cases where an etiological agent was definitely identified, has been reported only in the group from which the streptobacillus was isolated. In the 12 streptobacillus cases, the mortality has been 16 per cent.[1]

In case 1 of Blake, the patient had a systolic apical murmur, and died with ulcerative endocaritis. The patient in case 6 of Tunnicliff and Meyer died on the 17th day, and although one previous blood culture had been negative, a heart blood culture, post-mortem, revealed a streptothrix and Gram-negative coccobacilli; there also appeared to be a streptococcus present in smears of the bone

[1] Two additional deaths in the United States from infection with the streptobacillus have come to the attention of the writers. In one case an autopsy was performed by Dr. Ruben Z. Schulz of Boston, upon a patient who had had endocarditis. The other was a patient of Dr. L. A. M. Krause of Baltimore, and had a myocardial abscess. It is hoped that these two cases will be reported. In England, Stuart-Harris *et al.* (139) have reported a similar death from endocarditis due to a microörganism almost certainly identical with the streptobacillus.

marrow made at autopsy. Case 6 is difficult to evaluate, but certainly the streptobacillus was present.

Complications in streptobacillus cases, also seen in sodoku, have been generalized adenitis in cases 3 and 36. The patient in case 22 had photophobia, and in case 36, congestion of the conjunctivae.

Such signs and symptoms as severe sore throats, congestion of the pharyngeal mucous membranes, and cough with sore throat, seen not infrequently in streptobacillus cases, have been seldom mentioned in the sodoku cases. In only one streptobacillus case (# 18 of Mock and Morrow (96)), was a questionable pulmonary lesion (associated with cough) cited.

The most striking difference between the two types of illness has been the high incidence of joint involvement in the streptobacillus cases.

In case 10 of Dodd, the patient had severe joint pains, and in the 6 cases reported from 1932 to 1940 there was definite arthritis. In case 19 the patient had painful arthritis, in case 22, severe arthritis, in cases 31 and 32, mild polyarthritis, in case 35, arthritis which persisted for seven months, and in case 36, arthritis, and occasional twinges of pain in one hip, present a year later. Case 27 of the spirillary group can be definitely likened to these cases.

It must be remembered, however, that 25 per cent of the "Sodoku inoculata" cases of Hershfield *et al.* had arthritis as a complication. In this connection it is of interest that the observance of joint manifestations, as a part of the symptom complex of rat-bite fever, was not mentioned in any of the six "streptothrix" cases prior to the report of arthritis in the Haverhill epidemic in 1926 (Place, Sutton, and Willner (117)).

Once again, the question arises as to whether this recent occurrence of arthritis is actually new, or whether its sudden appearance is merely the result of a changing point of view. Enough similarities have occurred in the two types of cases, particularly when one considers the high incidence of arthritis in the *Spirillum minus* inoculated group of Hershfield, to caution against hailing arthritis as a complication which would differentiate the streptobacillus from the spirillary type of rat-bite fever.

It is of interest that in the Haverhill Fever cases in which the strepto-

bacillus (*Haverhillia multiformis*) was almost certainly ingested, gastrointestinal symptoms, such as nausea and vomiting, were present in a high percentage of the patients. Since these symptoms have occurred in both types of rat-bite fever, they cannot be employed to separate the streptobacillus from the spirillum infection.

It was found that in Tileston's case (2) (streptobacillus etiology), the patient developed dysphagia, vomiting, headache and fever (T—105°F.), 5–8 days following the bite. The onset of the illness in Dodd's case (10) was characterized by headache, vomiting, and a temperature of 103.8°F. On the other hand, in case 9 of Dembo *et al.*, in which the disease was allegedly due to a spirochaete seen in lymph fluid from the wound, the onset occurred one day after the bite, with diarrhea and vomiting. Case 24 of Gilkey and Dennie (44), from which a spirillum was obtained, had an immediate onset with vomiting and prostration, and with fever which was apparently steady for one week.

At this point further consideration will be given to Table II which includes 40 cases of rat-bite fever reported in the United States from 1931 through 1940. This table is a continuation of Bayne-Jones' (4) Table I which has summarized the cases from 1916 through 1930. Four additional cases previously overlooked have been added to Bayne-Jones' Table. The total number of American cases reported through 1940 has been 125. The attempt to obtain an etiological agent was made in 29 of the last 40 cases; animal inoculations or cultures, or both, were performed, and an agent was obtained in 21. Only 19 of these can be considered however, since two of the 21 were diagnosed by direct examination only, and must be omitted owing to insufficient proof.

If the figures are further broken down, it is found that in 11 cases animal inoculations alone were made, and in only four were the inoculations positive for *Spirillum minus*. In only five cases were cultures made, and in four of these the cultures were positive for the streptobacillus. There were 13 cases in which both animal inoculations and cultures were made, and 11 of these were positive for one or the other microörganism.

It becomes quite evident, then, that in those cases in which the investigators have made the most effort, the reward has been greatest in obtaining either the spirillum or the streptobacillus.

It is of interest to review the 19 cases of rat-bite fever reported from

1931 through 1940 (Table II) from which no causative agents were obtained, in order to see whether there were any characteristic features which might enable one to place them in one or the other category. They were all due to rodent bites: 17 due to rat-bite, and two due to mouse-bite. The serologic tests for syphilis were positive in six, negative in six, and unknown in seven cases. In one case (Caldwell and Templeton) both animal inoculations and blood cultures were performed; neither diagnostic method was used in nine cases; only animals were inoculated in seven cases, and in two cases only, were cultures made.

Of the entire group of 19 cases, at least four are worthy of brief comment.

In case 88 of Caldwell and Templeton (15) the onset of symptoms occurred two days after the rat-bite, with local inflammation in the wound, headache, nausea, vomiting, chills, and muscle pains in the legs. The Wassermann reaction was 4+ on one occasion and negative twice. Eight injections of arsenic were given and the patient had many febrile rises during the 42 days following the first treatment. The fever gradually subsided but there was persistence of muscle pains. Petechiae developed around the joints, and there were vesicles on palms and soles for many days. This certainly cannot be considered typical sodoku, but is quite typical for streptobacillus infection.

In case 95 of James' (56), symptoms appeared one week after rat-bite, with facial neuralgia and numerous febrile rises at three-day intervals. Thirty days after the bite, the patient developed an exacerbation of fever associated with abdominal tenderness and rigidity. There were two attacks of pain, tenderness, and swelling of the elbows, hands, and knee joints. The patient received three injections of neoarsphenamine resulting in subsidence of fever and disappearance of rash. However, five months later, there were still slight residual facial neuralgia and occasional attacks of "urticaria." This case presents features of both types of infection.

These two cases both had negative blood cultures, but the details of the media used are unknown.

Marshall's third patient (109)developed onset of symptoms with local inflammation one day after the rat-bite and a week later had chills and fever followed by vomiting and convulsions. There was a measles-like eruption on the face and extremities with some pustules and blebs, and blisters on palms and soles. On the 10th, 11th, and 12th days after the bite, there was swelling and pain in wrists and ankles. During the second week, there was apparently a daily febrile rise. In the 3rd week, stovarsol was given by mouth with one subsequent rise to 103°F.

Later, there was severe pain in the right shoulder and left knee. The patient developed a sore throat one month after the bite at which time the pharynx appeared normal. In this case, neither animals nor broth flasks were inoculated, but the symptomatology is much like the previous two cases.

Case 90 of Anderson and Spector (2) was a four-year old colored male who received multiple rat-bites on the right thumb and elbow. Onset of symptoms occurred seven days later with swelling of the arm and axilla, and with fever for one week. After four days of normal temperature, four typical relapses occurred at seven-day intervals. No rash was noted at any time. No serologic test for syphilis was reported. At the time of the expected 5th relapse on the 36th day, treatment was begun with quinine sulphate, and maintained for 11 days; thereafter, KI was given for 36 days more. There were no more relapses after beginning treatment. Nasal diphtheria developed as a complication 12 weeks after onset. There were no bone lesions by x-ray. Dark-field, Giemsa and Wright stained preparations of blood and serum from the swollen gland were all negative, as were intraperitoneal inoculations of white rats. Cultures of blood and serum from the gland were positive for a *sporothrix* during the 1st, 2nd, and 4th relapses, using anaerobic and aerobic dextrose and ascitic phosphate broth. This organism was agglutinated by the patient's serum in dilutions of 1/2560. So here, except for absence of rash, the clinical course is typical for sodoku; but response to "specific" treatment was prompt, agglutinins against the sporothrix were present in high dilution, and a spirillum was not found after careful search, although guinea pigs and mice should have been inoculated instead of rats. On the other hand, the case of cutaneous sporotricosis due to mouse-bite, reported by Moore and Davis (97), bore no resemblance to sodoku at all. Case 88 could not be considered typical of sodoku and case 95 presented features of both types of infection, while case 109 had a clinical course entirely compatible with streptobacillus infection. The clinical course of case 90, in which the isolation of a "sporothrix" was reported, could be considered "typical" of sodoku. In these four cases where bacteriological proof was either lacking or incomplete, one cannot rule out the possibility of streptobacillus infection.

The preceding comparative clinical analysis of rat-bite fever due to *Streptobacillus moniliformis* and sodoku (*Spirillum minus*), clearly indicates the overlapping of the main clinical features of the two types of infection (leucocytosis, rash, relapsing fever, positive serologic tests for syphilis, response to arsenical therapy[2] and arthritis[3]). It is apparent, therefore, that any attempt to draw sharp lines of differentiation between the streptobacillus and *Spirillum minus* forms of rat-bite fever, by means of clinical observations alone, must remain open to question at the present time.

[2] Definite, suggestive in streptobacillus infections.

[3] "Sodoku inoculata"—25 per cent arthritis (Hershfield (52)).

C. Report of Cases (Streptobacillus isolated)

Case 1. J. B. (J. H. H. Unit No. 182623), a 49-year old white farmer from Thurmont, Maryland, was admitted to the Johns Hopkins Hospital on September 25, 1939. The family history and past history were non-contributory.

On September 7th, while asleep, the patient was bitten on the left thumb by a rat. The wound bled profusely, was washed with cold water and bandaged but no antiseptic solution was applied. In several days the wound had healed completely without evidence of secondary infection. On the 3rd day following the rat-bite, he developed chills, fever, anorexia, and malaise. Swelling of the left knee appeared on the 8th day. Pink skin lesions were noted on the 16th day, together with laryngitis, cough, hoarseness and severe headache. He was admitted to the hospital on the 18th day of the illness, complaining of a severe sore throat, hoarseness, and pain in the left knee.

The patient was a well-developed middle-aged man, who presented evidence of some weight loss. The temperature was 102°F., pulse 100, respirations 20, and blood pressure 120/74. He complained of severe pain in the left knee whenever he was moved. He was very hoarse, and could not speak above a whisper. There were a few small pustules over each elbow, but no rash. The site of the rat-bite on the left thumb had healed completely without a scar. He complained of a severe sore throat, with the soreness extending down to the laryngeal region. Direct laryngoscopic examination revealed a number of abnormal findings shown in Fig. 1. The pharynx appeared only moderately injected. On the posterior part of the soft palate there was a small superficial ulcer covered with yellowish exudate. The teeth were in poor repair with numerous caries. At the angles of the jaw several small, non-tender glands were felt. The epitrochlear and axillary glands were barely palpable and equal in size on both sides. The heart and lungs were normal, and there was no abdominal tenderness nor were any masses felt. There was very marked swelling of the left lower leg with the greatest swelling in the region of the left knee joint. The slightest manipulation of this joint caused extreme pain. Below the knee the swelling was due to marked oedema, and pitting was noted over the entire lower leg, the ankles, and dorsal surface of the foot. The neurological examination was negative.

Examination of the blood revealed a slight degree of anemia. The red blood cell count was 4.07 million and the hemoglobin was 10.5 gms. The leucocyte count was 15,400 with 74 per cent polymorphonuclear cells. No abnormal cells or parasites were seen in the smear. The erythrocyte sedimentation rate was 36 mm./hr. corrected. The specific serologic test for syphilis revealed negative flocculation and Kline tests on two occasions, 9–25–39 and 1–15–40. Albumen (1+), and an occasional red blood cell, were present in the urine for the first three days in the hospital; thereafter, the urine was negative. No casts were seen at any time.

When the patient was first admitted to the hospital the history of rat-bite was not appreciated and numerous studies were carried out in an attempt to explain the illness. *Shigella paradysenteriae* (Flexner), *Salmonella suipestifer* and *Bru-*

cella abortus were not agglutinated by the patient's serum. The tuberculin test gave a mild positive reaction in 1:1000 dilution. In view of the appearance of the larynx, which suggested to the examiner the possibility of early tuberculous involvement, it was suggested that the arthritis of the left knee might also be tuberculous. Guinea pig inoculation and cultures on Petragnani's media were carried out on the joint fluid to attempt to verify this impression. (The results of three inoculations and cultures were later found to be negative.) Two blood cultures, two joint fluid cultures, and two cultures from the small lesions on the elbows, were carried out using the standard routine methods. These cultures were made aerobically, anaerobically and under CO_2, and all were reported negative. The urine culture was negative. The throat culture was negative for he-

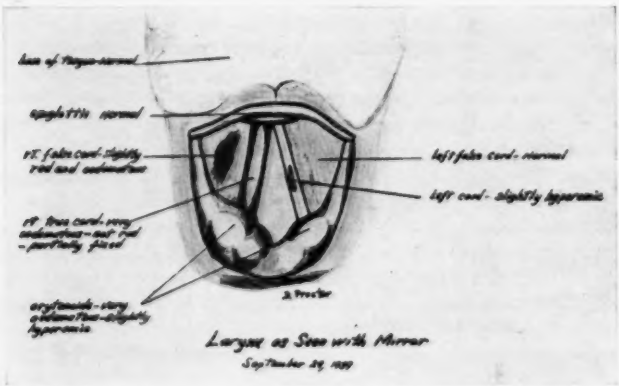

FIG. 1. *Case 1.* Drawing of larynx showing changes associated with acute phase of *Streptobacillus moniliformis* infection. Patient experienced a marked hoarseness and severe sore throat at this time. Note oedema of arytenoids and right vocal cord; also hyperemia of left cord, and right false cord.

molytic streptococci. The electrocardiogram was normal. The x-ray of the left knee revealed a "moderate amount of synovial thickening" on September 27th. Six weeks later, x-ray of the same joint revealed "loss of the joint space and destructive changes on the medial side of the joint."

For several weeks prior to the admission of this patient the writers had been attempting to recover organisms of the pleuropneumonia group from the joint fluids of patients with various types of unexplained arthritis. Accordingly, the joint fluid of this patient was cultured using special media and technics (see bacteriological methods). *Streptobacillus moniliformis* was easily recovered on several occasions (see Chart I) from the blood as well as the joint fluid by using the special pleuropneumonia culture methods. The patient had apparently been ashamed to mention that he had been bitten by a rat, until questioned directly

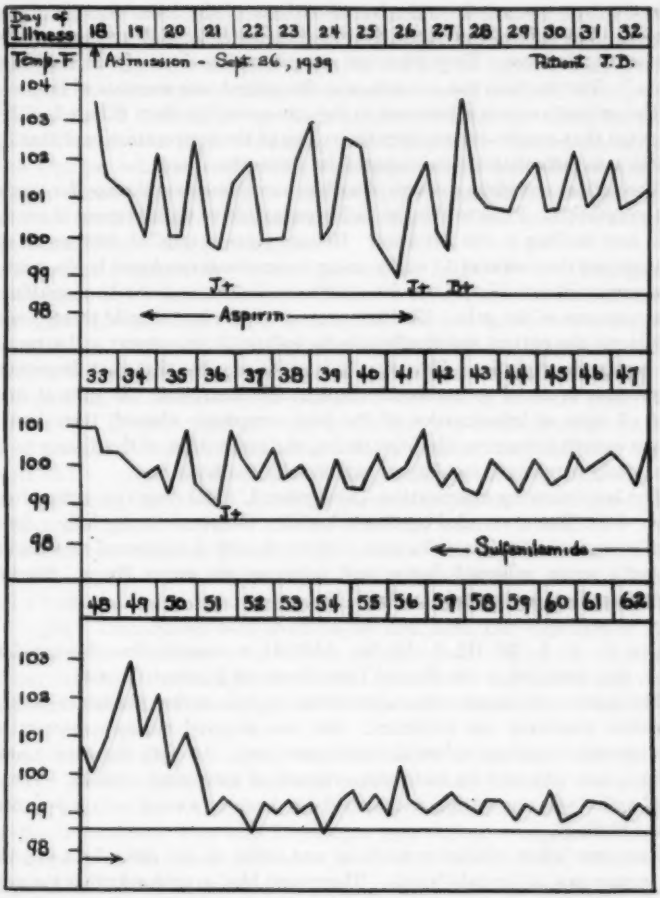

CHART I. *Case 1.* Male aet. 49. Temperature chart showing prolonged febrile course. Note the failure of aspirin to reduce temperature. Sulfanilamide produced no definite beneficial effect, and resulted in drug fever on the 8th day of treatment. *Streptobacillus moniliformis* was cultured on three occasions from the joint fluid (J+) and once from the blood (B+).

in the light of the bacteriological findings. He then gave us the complete details of the rat-bite.

On several occasions during the acute phase of the illness a transient mild

skin eruption was noted on the lateral surfaces of the lower chest, and the abdomen. The lesions were small, discrete, non-palpable, pink areas not exceeding 5 mm. in diameter. They blanched on pressure and closely resembled "rose spots." The eruption did not itch, and the patient was unaware of its presence.

The patient's course is best seen in the accompanying chart (Chart I). It may be noted that aspirin did not alter the course of the temperature, and that febrile reaction to sulfanilamide necessitated its discontinuation.

The patient was given a course of sodium aurothiomalate therapy for treatment of the arthritis. Prior to this, he had been unable to walk because of continued pain and swelling in the left knee. He was given a total of 1000 mgm. of the drug during the course of 13 weeks, using the methods employed in the treatment of rheumatoid arthritis (141), with resultant subsidence of the knee swelling and disappearance of the pain. The improvement following the gold therapy seemed definite as the patient was finally able to walk without support and returned to his regular farm duties. However, it is quite possible that such improvement might have occurred spontaneously without this treatment. In spite of the fact that all signs of inflammation of the joint completely cleared, there had been severe enough damage to the joint during the acute stage of the disease to cause apparently permanent inability to extend fully the lower leg.

The last follow-up examination, (November 4, 1941) over two years after the onset of the illness, revealed continued inability to extend the leg fully. Streptobacillus agglutination reactions were performed, with a number of samples of the patient's serum collected during and following the acute illness. Significant agglutinin titres were noted (see Table V).

Case 2. C. L. W. (H. L. H. No. A18444), a seven-weeks-old colored male baby, was admitted to the Harriet Lane Home on January 10, 1941.

The mother was found to have late latent syphilis during pregnancy, and antisyphilitic treatment was instituted. She had received nineteen arsenical, and two bismuth injections before the baby was born. At birth the baby appeared normal, and was entirely free from evidence of congenital syphilis. The neonatal period was uneventful, and normal development ensued with supplemented breast feedings.

Four days before admission the baby was bitten on the cheek by a rat, which the mother saw in the baby's crib. The wound bled so profusely that the mother brought the infant to the Johns Hopkins Hospital Accident Room, where the lesion was treated locally with antiseptic solutions; tetanus antitoxin was administered and the baby was taken home. During the next two days the baby was fretful, but did not develop acute symptoms until the day before admission when the mother noticed that he was feverish.

On admission the temperature was 39.7°C. and the weight was 4490 grams. The infant was irritable but otherwise did not appear acutely ill. The nutrition and development were good. A slight, fine exfoliation of the skin was noted over the trunk and the extremities, while the palms and soles were spared. The

fontanelles were not bulging, the neck was not stiff, and the neurological examination was negative. Several small erythematous macules were present on the right upper eyelid. On the right cheek, at the site of the bite there were five tiny linear puncture wounds clumped together in a small area, and surrounded by a fading zone of ecchymosis. A single puncture wound was noted on the left cheek. There was a slight reddening of the pharynx. The superficial lymph nodes were not enlarged. The lungs were clear. There was no cardiac enlargement and no murmurs were heard. The liver edge was palpable one finger breadth below the costal margin. The spleen was not felt. There was no swelling nor apparent tenderness of any of the joints.

The infant's temperature course can best be judged by the accompanying chart (Chart II), which shows the transition from a sustained temperature elevation, through a gradual return to nearly normal and finally to a period of relapsing fever. With each febrile episode, a generalized skin eruption developed. The rash was reddish, mottled and splotchy in appearance, and occurred in confluent patches, one to three cm. in diameter. The lesions were not visibly raised or palpable. The rash appeared first on the face and later spread to the entire body. Around the site of the rat-bite, there occurred induration of the soft tissues and a zone of erythema as well as generalized eruption coincident with each cyclic temperature rise. During the afebrile periods the rash completely disappeared.

The leucocyte count on the 4th, 29th, 43rd, and 45th days of illness was 23,700—30,550—15,800—8,550 respectively. During the first three weeks in the hospital a fairly marked anemia developed. The hemoglobin dropped from 12 gms. to 7.2 gms. Transfusions were given on the 31st, 37th, and 45th days of illness; a total amount of 220 cc. of blood was administered. There was no reaction, nor any apparent relationship between the time of the transfusion and the development of fever. The hemoglobin level was only temporarily elevated by the first two transfusions.

Four serologic tests for syphilis were performed during the febrile and convalescent periods, and a final test was made six weeks following discharge. In the first instance the complement fixation and flocculation reactions were determined; the remainder were only flocculation tests and gave negative results with the exception of the second one, taken on the 46th day of illness, which was reported as doubtful.

X-rays of the long bones were taken on the 7th, 17th, 28th, and 38th days of illness. No x-ray abnormalities were noted in the first plates, taken on the 7th day of illness. The remaining x-ray examinations did reveal a minimal degree of long bone periosteal proliferation in several areas. At no time during the baby's hospital stay was there further progression or regression of these periosteal changes.

The urine was normal at all times.

Streptobacillus moniliformis was recovered from the blood on the day of admission, the 4th day of illness. The special serum-enriched media and technics

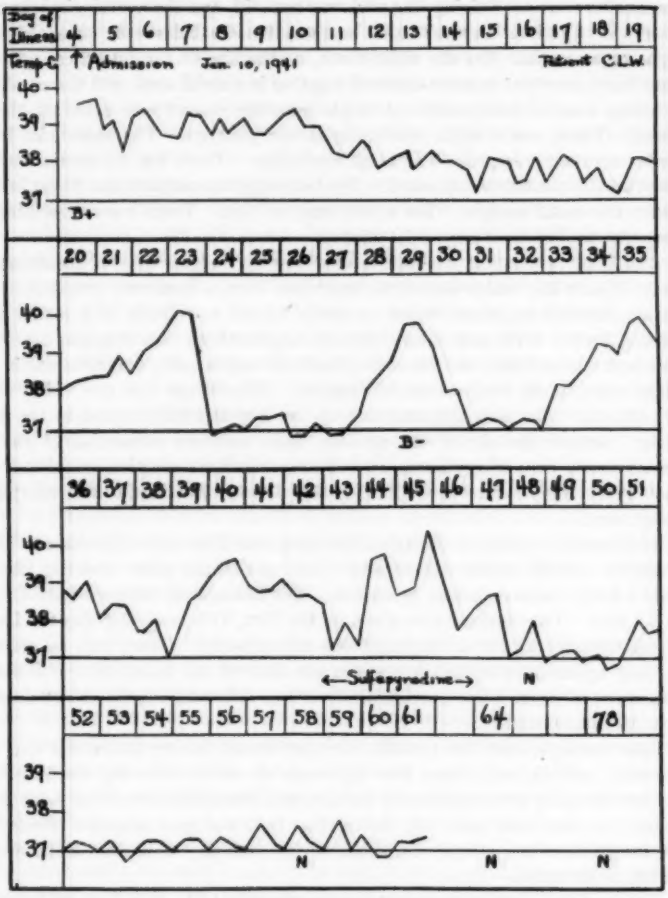

CHART II. *Case 2.* Male aet. 7 weeks. Temperature chart. The originally sustained temperature elevation gradually subsided during the first 3 weeks. Note later development of periodic febrile rises, suggesting the relapsing fever of sodoku. *Streptobacillus moniliformis* cultured from the blood (B+) on the 4th day. Culture negative (B−) on the 29th day, at the height of a febrile rise. Sulfapyridine failed to prevent a recurrence of temperature rise, but neoarsphenamine (N) appeared effective.

were used to good advantages in this case (see section on Culture Methods) as routine blood cultures were sterile. The development of the so-called "fluff balls" was slow, and it was only after subculturing that the growth of the microorganism became pronounced. Rabbit serum-enriched broth media gave the best growth in the initial culture. No growth occurred in the ascitic fluid broth culture. On the other hand, several typical streptobacillus colonies, which were detected by microscopic examination, developed on the ascitic fluid Dextrose Starch agar plates, and these were subcultured successfully. On the 20th day of illness, at a time when the temperature was at the high point of one of the cyclic swings, a second blood culture was taken, using the same technics which had been previously successful. On this occasion, however, the blood culture was sterile. Three other blood cultures, using routine blood culture media only, were sterile on the 29th, 44th, and 45th days of illness.

During the course of the illness the patient developed a high agglutinin titre against the streptobacillus recovered from the patient, as well as against the strain previously isolated in Case 1 (Table V).

In view of the cyclic temperature swings, the recurrence of skin eruption, both generalized and at the site of the rat-bite, with each febrile episode, and because of the negative cultures for the streptobacillus during this period, it was considered likely that the patient might have been infected with *Spirillum minus* as well as with the streptobacillus. The first part of the illness could have been due chiefly to the streptobacillus, and the second part, suggestive of sodoku, due to the spirillum. Accordingly, six Swiss mice were each inoculated intraperitoneally with 0.5 cc. of blood from the patient, and 2 guinea pigs were each given 1.0 cc. of blood intraperitoneally. Dark-field examination and smears stained for the spirillum were made daily on the tail blood of the mice from the 6th to the 14th day following inoculation. Similar studies were made on the peritoneal fluids of the mice, and the blood as well as the peritoneal fluid of the guinea pigs. Neither *Spirillum minus* nor any other spirillary forms were seen.

Between the 42nd and 47th days of illness the patient was treated with sulfapyridine. During these five days of sulfonamide therapy, the patient had one of the regular paroxysms of fever, and the temperature on this occasion reached the highest point during the entire hospital stay, namely, 40.4°C. On the 48th, 58th, 64th, and 78th days of illness, the baby was treated with neoarsphenamine, 50 mgm. on each occasion. Following the first injection of the arsenical, there were no further paroxysms of fever. All observers agreed that neoarsphenamine had favorably affected this patient's course.

Case 3. W. J. (H. L. H. No. A20118), a colored male infant aged three weeks was admitted to the Harriet Lane Home on April 30, 1941, because of an infected hand and fever following the bite of a rat.

There was no family history of syphilis or arthritis. The baby had been delivered spontaneously at full term, and at birth appeared strong and healthy, and weighed eight pounds and three ounces. Formula feedings were substituted

for breast feedings on the fourth day due to the mother's complication of post-partum fever. The baby remained well until the onset of the present illness.

Four days before admission the infant was bitten on the fourth finger of the right hand by a rat. Local treatment consisted solely of white vaseline rubbed on the wound. Two days later the baby began to cry, developed fever, and refused its feedings. The right hand at the site of the rat-bite appeared infected at this time.

On admission (the 5th day of illness) the baby was very irritable and appeared moderately ill. The temperature was 38.5°C. The fontanelles were open and not bulging, and the neck was not stiff. There was no evidence of a rash. The back of the right hand was badly infected in a region surrounding the site of the rat-bite, which was at the fourth metacarpal phalangeal joint. The region of infection was about 6 cm. in diameter, and extended to the middle of the fourth and fifth fingers, between these fingers and to the mid-portion of the back of the hand. The infected area was rough and crusted; there was some purulent material and a minimal degree of erythema. It did not have the appearance of a chancre. Over the lower end of the sternum there was a small, slightly reddened, barely palpable area which later became a point of considerable interest.

The conjunctivae were not infected, the ear drums were normal and there was no nasal discharge. The tonsils and pharynx were not infected. A moderately enlarged lymph node (about 2 cm. in diameter) was palpable in the right axilla. The superficial lymph nodes were not palpable elsewhere. There was no enlargement of the heart and the sounds were of good quality. A short soft systolic murmur was heard locally at the apex. The liver, spleen and kidneys were not felt. Examination of the extremities was of particular interest because of the presence of a very definite arthritis. There was redness and swelling of the left great toe, and similar involvement of the middle joint of the left third finger.

The temperature course can best be seen in chart III. The febrile response to a very servere infection was not great in this case. Nor was the temperature ever relapsing in type.

A summary of the leucocyte counts during the various stages of the baby's illness have been listed as follows: 27,000—27,100—25,000—17,300—12,200—9,800 on the 7th, 8th, 13th, 18th, 20th and 34th days of illness, respectively. The hemoglobin dropped from 14 gms. on the 5th day to 9.4 gms. on the 18th day, and finally rose to 10.6 gms. on the day of discharge (the 51st day of illness). At the time of admission the urine contained 1+ albumen. No red blood cells, white blood cells nor casts were seen in the urinary sediment.

Serologic tests for syphilis were made on the 8th, 15th, and 53rd days of illness. Both the flocculation and complement fixation tests were negative on all three occasions.

On the 9th day of illness the lesion (Fig. 2) over the sternum had become larger and slightly fluctuant. 1.0 cc. of thick yellowish pus was aspirated and the smear revealed 90 per cent polymorphonuclear and 10 per cent mononuclear cells. A Gram stained smear was entirely negative for any evidence of bacterial forms.

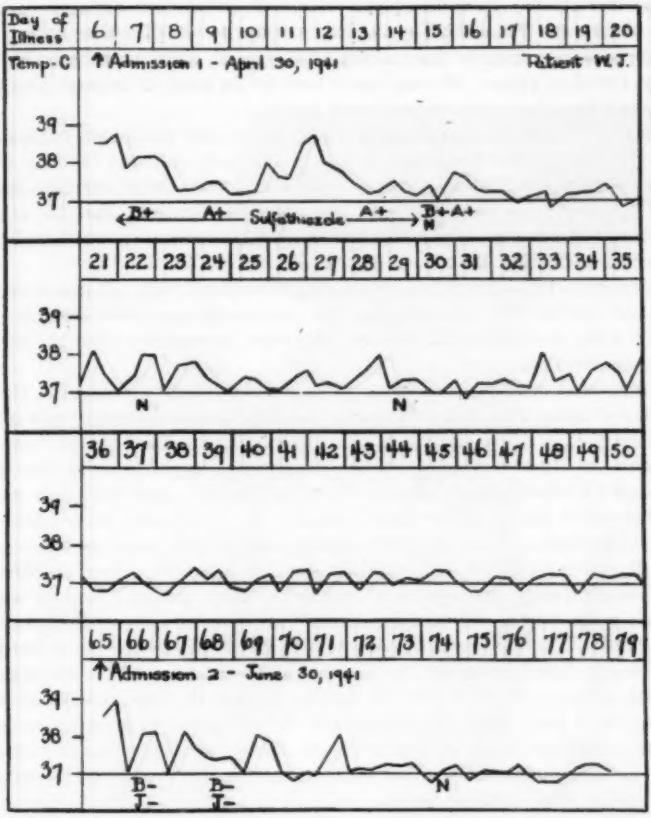

CHART III. *Case 3.* Male aet. 3 weeks. Temperature chart. Note the relatively mild febrile response in this case. *Streptobacillus moniliformis* was recovered from the blood (B+) on the 2nd day and again at the end of a 9 day course of sulfathiazole therapy. The microörganism was also recovered 3 times from the abscess (A+) during the period of sulfathiazole therapy showing the ineffectiveness of this form of treatment. Neoarsphenamine therapy is indicated by (N). Note the negative blood (B−) and joint fluid cultures (J−) at the time of the second admission.

Giemsa (dilute) and Wayson stained preparations were also negative for all forms suggesting the streptobacillus. On the other hand, by means of undiluted Giemsa stain, a number of extremely minute forms of bacillary type were demonstrated.

These more closely resembled pleuropneumonia forms than ordinary bacteria. The photograph (Plate III, Fig. 16) is an attempt to visualize these bodies. (It was necessary to magnify these bodies 1500 times in order to photograph them. They, therefore, appear 500 times larger than by the usual oil immersion magnifition, and thus give a false impression of size).

On the 17th day of illness the abscess over the sternum was opened and drained, and the affected area was compressed from this time until the 51st day of illness. No abnormalities were noted of the chest, heart, lungs, sternum, hands, or long bones. No X-ray evidence was obtained, therefore, that the abscess over the sternum had originated from periosteal involvement, which had been suspected clinically.

As noted in the Chart III, *Streptobacillus moniliformis* was cultured a number of times directly from this patient. The microörganism was isolated in pure culture twice from the blood, and on three separate occasions from the abscess over the sternum.

A number of mice were injected with blood and pus from the abscess. On the 7th day of illness three Swiss mice were inoculated intraperitoneally, with blood. Each received a 0.5 cc. inoculum. The streptobacillus was cultured from the peritoneal cavity of one of these mice. On the 13th day, four Swiss mice were inoculated intraperitoneally with pus from the abscess. Five days later mouse number one of this group developed arthritis. It was of great interest that the dark-field examination of the peritoneal exudate of this same mouse, on the 12th day following inoculation, revealed a spirillary organism; at least six different observers, including ourselves, were willing to accept this as *Spirillum minus*. At this point, had it not been for determined efforts to culture *Streptobacillus moniliformis*, this case would undoubtedly have been diagnosed as rat-bite fever due to *Spirillum minus*. In view of the previous recovery of the streptobacillus, we were willing to conclude for the moment that the patient had been infected with both the streptobacillus and the spirillum. Fortunately we were able to obtain from Dr. T. B. Turner a strain of true *Spirillum minus* for comparison. Examination of this spirillum made it immediately evident that we had not been dealing with *Spirillum minus*.

The baby was discharged well on the 51st day of illness only to be readmitted on the 65th day because of an apparent recrudescence of the former illness. Two days before this second hospital admission, the mother noticed that the baby did not use the right leg and she observed that the right knee was swollen and tender. She also noticed that purulent material was draining from the right ear.

On admission the temperature was 38.5°C. The physical examination revealed no evidence of skin eruption, and the site of the abscess over the sternum had healed completely. The skin over the right hand which was originally so severely infected at the site of the rat-bite had also healed. The inguinal and cervical nodes were palpable on this admission whereas the previously enlarged right axillary node was no longer felt. The left ear drum appeared normal. A moderate amount of greenish pus was draining from the right ear drum. There was red-

dening of the pharynx, although the tonsils appeared normal. Small white patches were observed along the gum margins. The heart was normal in size. The apical systolic murmur heard on the previous admission was no longer noted. The liver

FIG. 2. *Case 3.* Large subcutaneous abscess over the sternum. A pure culture of *Streptobacillus moniliformis* was recovered.

and spleen were not felt. The right knee joint was greatly swollen and hot. The swelling was periarticular as well as due to fluid within the joint proper.

On the day following admission (the 66th day of the disease), the pus from the right ear was cultured. Diphtheroids and *B. proteus* were recovered. No colonies of streptobacillus were seen. Fluid was aspirated from the right knee joint on

the 66th, 69th, and 81st days of the disease. The fluid was cloudy and yellowish in character, and clotted immediately after being withdrawn from the joint. Because of rapid clotting no accurate cell count could be obtained. Stained smears of the fluid revealed 90 per cent polymorphonuclear and 10 per cent mononuclear cells. Smears of the fluid stained with Gram, Giemsa, and Wayson stains, failed to reveal any recognizable bacterial forms. None of the elements previously seen in the Giemsa and Wayson stained preparation of the material from the abscess over the sternum, were present in the joint fluid smears.

On this second admission three different samples of the joint fluid and two blood samples were cultured for the streptobacillus using the special serum-enriched media. Of considerable theoretical interest, to be discussed in more detail elsewhere, was the fact that in none of these cultures was there any evidence of either the streptobacillus or the L_1 component by itself. The sterile cultures during this period of supposed recrudescence of the infection were of particular interest in view of the relative ease with which the streptobacillus was recovered during the first phase of the illness. Of the three strains of streptobacillus recovered from three patients, this strain (case 3) grew with the least difficulty.

Repeated attempts were made to visualize *Spirillum minus* in the joint fluid by dark-field examination. There was no evidence of any spirillary forms. That *Spirillum minus* might have caused the arthritis was unlikely in view of repeated injections of neoarsphenamine prior to the development of the arthritis. One of the characteristic features of typical sodoku has been the rapid and dramatic action of arsenical therapy in arresting the infection.

Various types of therapy were used in this case. As seen in the temperature chart sulfathiazole was entirely ineffective. The blood culture remained positive for *Streptobacillus moniliformis* during this course of treatment, and the abscess continued to enlarge steadily. Neoarsphenamine was used, but little can be judged regarding its effectiveness owing to the fact that the temperature had almost reached a normal level before the drug was administered. Furthermore, the abscess had been opened and drained, and although it continued to heal, it would be difficult to say that the neoarsphenamine had been responsible. It is of interest to note that the apparent recurrence of the infection occurred following neoarsphenamine therapy, and it is of further interest that cultures from the fluid as well as from the blood were uniformly negative for the streptobacillus at this time.

Gold therapy (sodium aurothiomalate) was finally used to treat the arthritis because of failure of the joint to improve spontaneously after 10 days. The swelling gradually subsided and the joint appeared normal five weeks after the institution of this therapy. As in case 1, the effectiveness of this form of therapy has been difficult to evaluate, since the joint might well have cleared spontaneously.

Samples of serum were obtained during and following this illness, and the result of a number of streptobacillus agglutination reactions with this serum have been summarized in Table V. It is to be noted that the streptobacillus agglutinin titre in this patient reached the highest level we have yet seen.

D. Summary and Analysis of Case Reports

Streptobacillus moniliformis was recovered from each of these cases, by the use of special culture media and technics (Section VII). Parallel cultures in routine blood culture media, were uniformly sterile in each case. The attempt was made to recover *Spirillum minus* as well as *Streptobacillus moniliformis* from cases 2 and 3, by the inoculation of mice and guinea pigs with blood from each case, and pus from the abscess of case 3. Repeated dark-field examinations of the peritoneal exudates of the inoculated animals failed to reveal *Spirillum minus*. One spiral microörganism, seen in a mouse inoculated with pus from case 3, was proven not to be *Spirillum minus* by comparison with a known strain of the latter.

The streptobacillus was agglutinated in significant titre by sera from each of the three cases (Table V); in each there was an increase in the agglutinin level during the course of the illness, and the highest titre reached was 1:5120 in case 3.

Arthritis was present in cases 1 and 3. The streptobacillus was recovered twice from the affected joint in case 1, and permanent joint changes developed in this case. To our knowledge this is the first recorded instance of rat-bite fever due to *Streptobacillus moniliformis* in which there have been permanent joint changes. Place and Sutton (116) have reported, however, such permanent joint damage in several cases of Haverhill Fever. Apparently two types of arthritis were seen in case 3. The first type was noted during the early bacteremic phase of the illness, at which time two small joints (finger and toe) were involved. Reddening, slight swelling, and local heat were noted (no x-ray changes); no fluid was obtainable for culture. It seemed reasonable to assume that this first type of arthritis represented the usual metastatic form seen in patients with this variety of infection. The second type of arthritis was sterile and developed in a previously unaffected joint following a two-week period of freedom from symptoms. This latter form of arthritis aroused considerable theoretical interest because of its similarity to many of the unexplained sterile arthritides. It should be noted that the patient's streptobacillus agglutinin titre had reached the very high level of 1:5120, at the time of the recurrence of fever and the development of the sterile arthritis. The possibility arose, therefore, that this late

TABLE V

Streptobacillus moniliformis Agglutination Reactions

(Cases from which Streptobacillus was isolated)

CASE NO. INITIALS	DATE		AGGLUTININ TITRES										
	Rat-bite	Serum obtained	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Saline control
1 J. B.	9- 7-39	10- 6-39	++++	++++	++++	++++	++++	+++	++	+	−	−	−
		10-13-39	++++	++++	++++	++++	++++	++++	++	+	−	−	−
		10-20-39	++++	++++	++++	++++	++++	++++	+++	++	−	−	−
		11- 1-39	++++	++++	++++	++++	++++	++++	+++	++	−	−	−
		11-22-39	++++	++++	++++	++++	++++	++++	+++	++	−	−	−
		1-31-40	++++	++++	++++	++++	++++	++++	+	−	−	−	−
		2-21-40	++++	++++	++++	++++	++++	++++	+	−	−	−	−
		11- 4-41	+++	++	+	+	±	−	−	−	−	−	−
2 C. L. W.	1- 6-41	1-16-41	++	++	+	+	−	−	−	−	−	−	−
		1-24-41	++++	+++	++	++	++	+	−	−	−	−	−
		1-29-41	++++	++++	+++	+++	+++	+++	−	−	−	−	−
		2- 7-41	++++	++++	++++	++++	++++	+++	+	−	−	−	−
		2-12-41	++++	++++	++++	++++	+++	++	++	++	++	−	−
		4-25-41	++	++	++	++	+	−	−	−	−	−	−
		6- 6-41	+++	+++	+++	++	+	−	−	−	−	−	−
		7-18-41	+++	++	+	−	−	−	−	−	−	−	−
3 W. J.	4-26-41	5- 1-41	++++	++	+	−	−	−	−	−	−	−	−
		5- 9-41	++++	++++	+++	++	+	±	±	±	−	−	−
		5-20-41	++++	++++	+++	++	++	++	++	+	−	−	−
		6-18-41	++++	++++	++++	++	++	+	+	+	+	+	−
		7- 2-41	++++	++++	++++	++++	+++	+++	+++	+++	++	++	−

form of sterile arthritis might have been due to a hyperergic (antigen-antibody) reaction. For many years the theory of hyperergy as an explanation for the various sterile arthritides has been held by numerous investigators (62, 63, 64, 65, 90, 131, 140, 156). The writers believe that this case may furnish a valuable basis from which to pursue the experimental production of sterile hyperergic arthritis. We are not aware of any studies which have made use of the streptobacillus, the L_1 pleuropneumonia-like microörganism, or any other member of the pleuropneumonia group, from this point of view.

The temperature course in cases 1 and 3 was irregular and at no time suggestive of the typical relapsing fever of sodoku. On the other hand, case 2 developed three cyclic rises in temperature at six-day intervals beginning on the 23rd day of illness, prior to which time the temperature had gradually subsided from an originally sustained level. Two additional temperature rises occurred with their peaks at five-day intervals. These temperature swings were suggestive of those seen in sodoku.

A rash was noted in the first two cases, but not observed in the third. In case 1, the lesions resembled "rose spots" of typhoid fever, and they reappeared on several occasions during the acute phase of the illness. In case 2 the rash was much more marked; the lesions were confluent rather than discrete, they presented a reddish, mottled appearance, were not visibly or palpably raised, and the borders were poorly defined. This skin eruption occurred during each episode of relapsing fever. There was also return of inflammation at the site of the previously healed rat-bite. The recurrence of the rash, and the inflammation at the site of the bite, were of particular interest in this case of known streptobacillus infection, in view of the fact that this has been described as a characteristic feature of sodoku.

Severe sore throat and laryngitis occurred in case 1. The laryngeal changes consisted of oedema of the arytenoids, swelling and partial fixation of the right true vocal cord, and areas of hyperemia of the left true vocal cord and the right false cord. It was the impression of the laryngologist who observed these changes prior to the definite diagnosis of the disease by culture, that the patient was suffering from early tuberculous laryngitis.

The effect of therapy in these cases, as in so many others in the past,

has been difficult to evaluate although certain impressions have been gained. In case 1 aspirin was administered for one week to ascertain whether there was an effect in this infection similar to that of aspirin in rheumatic fever. No definite effect could be noted. A period of therapy was attempted with the sulfonamide drugs; *i.e.*, sulfanilamide, sulfapyridine, and sulfathiazole, each patient receiving a different one of these three. In case 1, the patient was given one week of sulfanilamide therapy during which the temperature gradually rose and finally reached 103°F. (apparently drug fever). Treatment was discontinued. In case 2, one of the typical cyclic temperature rises occurred during a five-day period of sulfapyridine therapy. In case 3, there was convincing evidence of the therapeutic failure of sulfathiazole which was administered for a period of nine days. Two blood cultures, one taken on the second day of therapy, and one at the time the drug was discontinued, were both positive for *Streptobacillus moniliformis*. Cultures of the abscess were likewise positive for the microörganism on the fourth and eighth days.

A single injection of neoarsphenamine in case 2 was followed by a dramatic cessation of the relapsing fever. In case 3 the temperature was returning to normal at the time neoarsphenamine therapy was begun. In spite of three arsenical injections, the patient developed a recrudescence of symptoms with fever and arthritis. However, blood and joint fluid cultures were sterile at this time. Arsenical therapy was not employed in case 1.

A gold preparation (sodium aurothiomalate) was used to treat the arthritis in cases 1 and 3. In case 1, at the time gold therapy was instituted, the joint involvement had reached a chronic, incapacitating, apparently fixed state. Following a course of gold therapy in doses similar to those used for the treatment of rheumatoid arthritis (47, 141) the joint gradually improved, the pain disappeared, and the patient was able to resume his farm work. In case 3 the arthritis cleared up completely following gold therapy. It is, of course, impossible to say whether or not these favorable changes were purely coincidental with the gold therapy. These cases are the only two recorded in which gold therapy has been used to treat arthritis due to *Streptobacillus moniliformis*, and the future alone can prove its clinical efficacy in this type of infection. On the other hand, there is definite experimental evidence that certain gold compounds do have potent, specific, chemo-

therapeutic action in preventing animal infection produced by *Streptobacillus moniliformis* (50, 13) as well as the arthritogenic members of the pleuropneumonia group (32, 126, 127). Therefore, it is not unreasonable to assume that the gold therapy did play a rôle in the recovery of these patients.

E. Presumptive Diagnosis of Additional Cases by Streptobacillus Agglutination Reactions

The development of a simple method for preparing a diffuse streptobacillus antigen, and the demonstration of apparently specific agglutination reactions with this antigen (Section VII) has encouraged the writers to examine additional cases of rat-bite fever for the presence of streptobacillus antibodies. Five cases of rat-bite fever from which no specific microörganism was recovered have been included in this group, because of the demonstration in each instance of streptobacillus agglutinins in significant titre. There is good evidence, both on clinical and serologic grounds, that these cases were definite examples of *Streptobacillus moniliformis* infection; however, we have preferred to consider them under the heading of *presumptive diagnosis* because of the absence of cultural proof. Two of these cases came to our attention through examination of the hospital records. One was seen in 1934 and the other in 1938. There was no mention in the records of the possibility of streptobacillus infection, none of the streptobacillus culture methods were employed, and the illnesses remained unexplained. In the last three cases, some of the specific culture methods were used, but the streptobacillus was not recovered.

Case 4. H. W. (H. L. H. No. 85577), a 10-month-old white male baby, was bitten on the right hand by a rat on January 13, 1934. Four days later a generalized skin rash appeared, and a physician described it as typical of measles. On January 19, the infant was again bitten by a rat, this time on the left cheek. During the next three days he became feverish and weak, and on January 22, was admitted to the Harriet Lane Home.

The physical examination revealed scars from the bites on the cheek and hand. The wound on the hand had healed completely, whereas the cheek appeared swollen There were a number of minute purplish papules on the soles of the feet. The dorsal surfaces of the hands and feet were swollen, but there was no pitting. There was no enlargement of the superficial lymph nodes. STS was negative. The blood leucocyte count was 35,950.

The temperature remained elevated for two weeks, and the febrile period was

terminated abruptly following a final, single spike in temperature. On January 23rd the swollen area on the cheek broke down and discharged thick brownish yellow pus. Smears of the pus stained with a number of different stains were negative for bacteria and spiral microörganisms. "No spirochetes were seen in the dark-field examination of the pus." A Gram-negative bacillus (not identified) was recovered from the blood. Arsenical therapy was not administered.

In May, 1941, over six years following the rat-bite, the child was asked to return, in order to obtain serum for *Streptobacillus moniliformis* agglutination reactions. The test of the serum revealed a definitely positive, although low agglutinin titre against this microörganism (see Table VI).

Case 5. O. H. (H. L. H. No. A 7990), a month-old colored female baby, was admitted to the Harriet Lane Home on August 22, 1938. One week before admission a rat bit the baby on the left upper eye lid. This was followed by fever and nasal discharge five days later. The physical examination revealed a tiny, malnourished baby with a congenital dislocation of the left hip. A fading macular skin eruption was present on the feet. The laceration on the eye lid had almost completely healed. There was no enlargement of the lymph nodes or spleen. The liver was slightly enlarged. There was a considerable degree of anemia, and the leucocyte count was 31,200. Repeated serologic tests for syphilis were all negative except for one doubtful flocculation test. X-ray examinations of the extremities revealed punched out areas in the cortices of the long bones, which were considered to be due to syphilis. The STS on the father's blood revealed a positive flocculation and complement fixation reaction whereas the mother's blood was negative. A moderate, irregular, gradually subsiding febrile course persisted for two weeks, and, as in case 4, was terminated by a single isolated temperature spike. No mention was made of a possible *Streptobacillus moniliformis* infection in this case, and consequently, there was no specific culture attempt to recover this microörganism. Congenital syphilis was considered the most likely diagnostic possibility in spite of absent serologic confirmation, and antisyphilitic treatment was instituted. Twenty-six injections of sulfarsphenamine, and twenty-five of bismuth were administered during the ensuing year. Following this treatment the x-ray appearance of the long bones had become essentially normal. In February and April, 1941, approximately 2½ years following the rat-bite, serum was obtained for *Streptobacillus moniliformis* agglutination studies. As noted in Table VI the patient had, even at this late date, a high streptobacillus agglutinin titre.

Case 6. D. H. (H. L. H. No. A 13398), a two-month-old colored female baby, was seen in the Out-Patient Department of the Harriet Lane Home on November 1, 1929. Two weeks before, the infant had been bitten on the head by a rat, and the wound was immediately treated with mercurochrome and vaseline. For several days before being seen the baby had vomited frequently, and kept its legs drawn up apparently because of abdominal pain. Physical examination revealed small

vesicles on the upper lip, and on the scalp at the site of the bite, crusted lesions with small pustules at their bases. There was no generalized skin eruption or enlargement of the superficial lymph nodes. The baby cried when moved, though there were no visible abnormalities of the joints. Greenish-yellow purulent material discharged from the vaginal orifice, but no gonococci were noted in the stained smear. The child was referred to the Baltimore City Hospital where it was admitted (Nov. 1, 1939). The temperature was 101°F. The legs were held in a flexed position, and they could not be easily extended. The remainder of the physical examination was the same as that noted above. The laboratory findings were as follows: The blood leucocyte count was 16,250 and the Hgb. 9.8 gms. The urine contained a trace of albumen. The tuberculin (1.0 mgm.) and Wassermann examinations were negative. "Agglutination reactions (usual antigens) were negative." Three blood cultures were taken during the acute febrile illness. Two of these were carried out by the routine methods. A third culture attempt was made by us using the special streptobacillus culture methods, the blood being obtained from the Baltimore City Hospital on November 4, 1941 (17th day following rat-bite). All cultures were negative. Five mice and two guinea pigs had been inoculated intraperitoneally with the patient's blood. According to the Baltimore City Hospital report, "Smears from mouse blood all negative for spirochetes. No evidence of Weils' disease in guinea pigs. Dark-field examinations of patient's blood negative for spirochetes."

The child's temperature ranged between 101° and 104°F. "The legs were kept flexed, and the baby cried as if in pain, so that peritonitis was suspected; however, there were never any other symptoms suggestive of it." On November 3, a fine maculo-papular rash developed on the feet and lower legs. This soon disappeared. On November 5 (22 days after the rat-bite) the finger joints became red and enlarged. The signs of arthritis subsided two days later. Arsenical therapy was not employed. The temperature became normal on November 17 (about one month after the bite). The final diagnosis was "Fever of unknown origin. Rat-bite fever questionable."

On May 2, 1941, at our request the child was brought to Johns Hopkins Hospital, Out-Patient Department, where the mother stated that there had been no recurrence of arthritis during the interval. Serum was taken for the first time (1½ years following the bite), for streptobacillus agglutination studies. We were surprised to find a specific agglutinin titre of 1/320 (see Table VI).

Case 7. J. Y.[4], a 21-year-old white male from the National Institute of Health, Bethesda, Maryland, was bitten by a laboratory rat on April 19, 1941. Chilly sensations, headache, nausea, and vomiting developed three days later. On April 23rd, he was admitted to the United States Marine Hospital in Baltimore, Maryland. The physical examination revealed several enlarged cervical lymph

[4] This case has been included in a recent report by Dr. C. L. Larson, *Public Health Reports*, Vol. 56, No. 40, pp. 1961–69.

nodes, slight abdominal tenderness, and a barely palpable liver edge. There was no skin eruption, and the joints were normal. The admission leucocyte count was 6,950 and the highest count during the illness was 8,400. Repeated serologic tests for syphilis (flocculation and complement fixation) were negative on admission, positive two months later, and negative at the end of 4½ months. The temperature which was 38.6°C. on April 23, returned to normal in two days, but rose again to 38.8°C. on April 27th. With the second temperature rise the patient's condition suddenly changed. He became irrational, and developed an erythematous skin eruption on the wrists, forearms, and ankles. The temperature returned to normal the next day, and again rose to 38.8°C. on May 5, at which time he developed generalized joint pains. The right elbow, the most severely involved joint, was swollen and tender, but not reddened. From this point on, the patient improved slowly, the arthritis gradually subsided, and a persistent low-grade fever finally disappeared on July 1. Arsenical therapy was not employed. During convalescence the patient complained of sore throat which had not been present earlier in the illness. On May 3, we were asked to see the patient by Dr. E. T. Ceder of the Marine Hospital, and at this time we received blood for special streptobacillus cultures, and serum for agglutinations. A number of cultures, made with a variety of sera added to the basic media, were all negative. The streptobacillus agglutination reaction was, however, positive in significant titre as noted in Table VI. After another 2½ months the agglutinin titre had fallen to a level practically within normal limits.

Case 8. C. N. (H. L. H. No. A-21317), a four-month-old white male baby, was admitted to the Harriet Lane Home on August 8, 1941. Two months previously, the baby had been bitten on two fingers of the left hand by a rat. One week later he developed a generalized skin eruption which was diagnosed measles by a physician. The fingers became swollen a short while after the bite and finally discharged purulent material. Five weeks following the bite the baby began to keep the right leg drawn up and finally the mother noticed that the right knee was larger than the left. The physical examination revealed a very pale undernourished child. The superficial lymph nodes were enlarged on the left side of the neck and in the inguinal region. The spleen and the liver were both easily palpable. The right knee joint was swollen, and the hamstring muscles on the right were held in tight contraction, preventing full extension of the leg. There was a mild degree of anemia. The leucocyte count was 14,350. A blood culture on August 9 was negative. STS was negative. X-ray of the right knee revealed soft tissue swelling, but no evidence of bone destruction. Six days after admission an extensive skin eruption developed on the face and arms. The lesions were a mixture of small pustules and blotchy, red spots. There was very little fever at any time during the 2½ weeks of hospitalization. The swelling of the knee joint gradually subsided, and traction on the extremity resulted finally in relaxation of the hamstrings and straightening of the affected leg. Arsenical therapy was not employed. At intervals following the rat-bite, four samples of serum were obtained

for streptobacillus agglutination studies. The first sample of serum obtained on August 19 showed no evidence of specific agglutinins even in the lowest titres, whereas significantly high titres were reached with the later sera. The details of these agglutinations have been recorded in Table VI.

In Table VII the main clinical features of these five cases have been summarized and compared with cases 1, 2, and 3, in which streptobacillus infection was proven by culture. The total number of cases is small, yet there is an interesting parallel in the two groups in the incidence of relapsing fever, rash, arthritis, leucocytosis, and the presence of streptobacillus agglutinins. In only four of the eight cases was a search made for *Spirillum minus*—animal inoculation in three and direct examination in one. No spirilla were found. We do not think that conclusions can be drawn as to the possible presence or absence of *Spirillum minus* in these cases, even where the search for spirillary forms was made. We now realize that one must repeatedly examine the peritoneal exudate as well as the blood of mice for at least a month before discarding them as negative. Such a prolonged search was not carried out here. On the other hand, it is a very striking fact that specific streptobacillus antibodies were demonstrated in the blood serum of all of the eight cases of rat-bite fever that have come to our attention.

BIOLOGY OF THE STREPTOBACILLUS MONILIFORMIS

In order to appreciate fully the biological complexities of this microorganism, it is necessary to become familiar with the highly important discovery of Klieneberger (66) in 1935. Her researches on the streptobacillus directed attention to the very interesting group of filter-passing, virus-like microörganisms, known as the pleuropneumonia group.

The streptobacillus was first isolated by Schottmüller (129) in Germany in 1914, and was studied subsequently by a number of distinguished investigators; however, it remained for Klieneberger working in England to demonstrate clearly that colonies of this microörganism contained two morphologically distinct elements. By the use of specialized technics, she observed microscopic colonies, which were present in each of a number of streptobacillus strains recovered from rats. Klieneberger isolated these tiny colonies in pure culture, and

TABLE VI

Streptobacillus moniliformis Agglutination Reactions

(Presumptive diagnosis group)

CASE NO. INITIALS	DATE		AGGLUTININ TITRES									
	Rat-bite	Serum obtained	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Saline control
4 H. W.	1-13-34 1-19-34	5-20-41	+++	+++	++	++	−	−	−	−	−	−
5 O. H.	8-15-38	2- 7-41	++++	++++	++++	++++	++++	+++	+	+	−	−
		4- 1-41	++++	++++	+++	++	++	+	+	−	−	−
6 D. H.	10-17-39	5- 2-41	++++	++++	++++	++++	+++	++	−	−	−	−
		7-14-41	+++	+++	++	±	−	−	−	−	−	−
7 J. Y.	4-19-41	5- 3-40	+++	+++	+++	+++	++	±	−	−	−	−
		7-14-41	+++	+++	++	±	−	−	−	−	−	−
8 C. N.	6- 8-41	8-19-41	−	−	−	−	−	−	−	−	−	−
		9- 3-41	++++	++++	++++	+++	++	++	+	+	−	−
		9-23-41	+++	+++	++	++	+	−	−	−	−	−
		11- 4-41	++++	++++	++++	+++	++	+	−	−	−	−

TABLE VII

Analysis of Present Case Reports

TABLE VII

Analysis of Present Case Reports

CASE NO.	INITIALS	YEAR	AGE	SEX	C	RELAPSING FEVER	RASH	ARTHRITIS	LEUCOCYTE COUNT	STS*	STREPTOBACILLUS AGGLUTINATION (MAXIMUM TITRES)	THERAPY	RESPONSE TO THERAPY
Streptobacillus isolated													
1	J. B.	1939	49	M	W	0	+	+	15,000	0	1/1280	Sulfanilamide	None
												Gold (arthritis)	Good (?)
2	C. W.	1941	7 wks.	M	C	+	+	0	30,500	Doubtful floc.	1/2560	Sulfapyridine	None
												Neoarsphenamine	Excellent (?)
3	W. J.	1941	3 wks.	M	C	0	0	+	27,000	0	1/5120	Sulfathiazole	None
												Neoarsphenamine	Fair (?)
												Gold (arthritis)	Good (?)
Presumptive diagnosis													
4	H. W.	1934	10 mo.	M	W	0	+	0	35,950	0	1/80†		
5	O. H.	1937	1 mo.	F	C	0	+	0	31,200	Doubtful floc.	1/1280‡	Arsenical	Fair (?)
6	D. H.	1939	2 mo.	F	C	0	+	+	16,250	0	1/320		
7	J. Y.	1941	21	M	W	+	+	+	8,400	4+ floc.			
										4+ comp. fix.	1/160		
8	C. N.	1941	4 mo.	M	W	0	+	+	14,350	0	1/1280		
						25%	88%	62%		12% (positive)			
										37% (pos. + doubtful)			

Specific streptobacillus culture methods employed in cases 1, 2, 3, 6, 7, 8; streptobacillus recovered from cases 1, 2, 3 only.

Attempt to recover *Spirillum minus*—animal inoculation—Cases 2, 3; attempt to visualize *Spirillum minus*—direct dark-field pus—Cases 3, 4: results, no spirilla seen.

Under heading "Response to Therapy," the results "excellent," "good," and "fair" have been questioned, as it was almost impossible to say whether or not comparable clearing of symptoms would have occurred without specific therapy.

Source of infection each case—rat-bite.

All cases from State of Maryland.

* STS—Serologic Test for Syphilis.

† Persistence of agglutinins (low titre), 7 years after bite.

‡ Persistence of agglutinins (high titre), 2½ years after bite.

made detailed studies of their structure. She originally believed, and has maintained the view, in spite of considerable opposition, that these minute colonies were *symbionts*, and not *variants* of the streptobacillus. In attempting to find a proper nomenclature for these forms, Klieneberger noted their close similarity (minute size, growth requirements, staining characteristics and general morphology) to a microörganism, pathogenic for cattle, known as *Pleuropneumonia bovis* (106, 107). (This microörganism although able to multiply in cell-free media, has the distinction of being classified with the viruses, because of its ability to pass through filters of pore size sufficiently small to withhold the smallest known bacteria.) Klieneberger, therefore, named these streptobacillus *symbionts*, "pleuropneumonia-like microörganisms," and to obviate this cumbersome term, has referred to them as L forms. She soon discovered, in culturing certain lesions in rats and other animals, morphologically similar but immunologically distinct L forms which were unassociated with either the streptobacillus or any other microörganism. She separated these L forms by utilizing their ability to produce specific agglutinins, and she attached separate designations to each, such as L_1, (L_2), L_3, L_4, etc. Sabin (125) uses the letters of the alphabet to designate the various forms, such as Group A, Group B, etc. Needless to say, a single generally acceptable method of classification would greatly simplify matters and would avoid much confusion in the future.

We are chiefly concerned in this communication with the L_1 forms, which are the only members of the pleuropneumonia group found in association with the streptobacillus. We shall refer to the L_1 form as the L_1 *component* of the streptobacillus, since *component* does not imply the specific rôle indicated by either of the terms *symbiont* or *variant*. The symbiont-variant question is still undecided.

Our knowledge of the complex biology of the streptobacillus is recent and incomplete, and future observations may alter the current beliefs concerning the etiology of rat-bite fever. For this reason, we shall devote considerable space to a discussion of our own experience with this microörganism.

A. Culture Methods and Growth Requirements

The methods for the cultivation and microscopic study of the streptobacillus represent a definite departure from our usual concepts of

complete clinical bacteriological methods. The growth requirements of the streptobacillus and the L_1 component are the same as those of the pleuropneumonia group in general (66, 24, 67). The culture media and the technics employed are not complex and yet without strict adherence to a few definite rules, failure may well result. Furthermore, these methods are not used in routine clinical bacteriology, which fact might readily explain failures to recover this microörganism more frequently from cases of rat-bite fever. For example, in each of the writers' three cases, routine culture methods as well as the specialized methods were employed in the attempt to recover *Streptobacillus moniliformis* from the blood of the patients. In each instance, the clinical bacteriological laboratory reported negative blood cultures whereas parallel cultures in which the special technics were used yielded abundant growth of the microörganism.

Case 3 was of particular interest because of the finding of occasional motile spiral microörganisms in the dark-field preparation of the peritoneal exudate of one of four mice injected intraperitoneally with pus from the abscess. Twelve uninoculated normal mice of the same stock were similarly examined for the presence of spirilla and none was found. It was later ascertained that the spirillary forms recovered from the inoculated mouse were morphologically unlike *Spirillum minus*; numerous similar forms were also found in the feces of normal mice. It becomes immediately apparent, that had we employed only routine blood culture technics and also failed to compare the spirillary forms found with a known *Spirillum minus*, this case might well have been diagnosed sodoku, and not *Streptobacillus moniliformis* infection.

We have employed two types of basic media, Tryptose Phosphate and Dextrose Starch broth and agar.[5] A pH level of 7.6 was preferred, although adequate growth occurred throughout a pH range of 7.0 to 8.0. The factor of pH was of greater importance in preparing a diffuse antigen for purposes of agglutination. This will be referred to later.

As in the case of the pleuropneumonia group in general the most important single constituent in the media has been some form of serum protein. We have used a variety of different sera; horse serum gave the best results, although rabbit serum, ascitic fluid, beef serum and swine serum were used with success. Ascitic fluid was employed more extensively than any of the other sera mentioned, because

[5] These media have been kindly supplied to us for experimental purposes by Dr. E. G. Dunham of the Difco Laboratories.

of its easy availability and the quantity used. Furthermore, in no instance have we failed to recover the streptobacillus in the ascitic fluid enriched cultures, when growth was obtained with any of the other sera in the media. Not all samples of ascitic fluid have given the same results. Those with the highest protein content (over 3 gms. per 100 cc.) have given the best growth. All serum and ascitic fluid has been Seitz filtered and tubed through a closed system, incubated at 37.5°C. for 24 hours to test sterility, and kept in the ice-box until used in the media.

Various concentrations of ascitic fluid and serum have been employed. In the earlier work 33 per cent serum broth was used routinely. More recently 20 per cent serum and ascitic fluid concentrations have been used with equally good results. The best growth was obtained when the serum had been added to the basic media just prior to inoculation. As a matter of practical routine, culture tubes containing 4 cc. of the basic medium were stored in the ice-box until ready for use, at which time 1–2 cc. of ascitic fluid or serum were added.

Attention to details proved to be most important when the agar plate was employed. For example, the consistency of the agar was found to influence the growth. The softest possible agar, at the same time firm enough to remain in place, (about 1.25 per cent agar in the basic media), was the most satisfactory. A second important factor was the use of clear media and clear Petri dishes (preferably Pyrex), which permitted examination of the streptobacillus and L_1 colonies directly through the agar (Petri dish inverted) with the low power lens of an ordinary microscope. By this method the characteristic appearance of streptobacillus growth was clearly differentiated from other types of bacterial colonies. A further advantage was the elimination of the surface contaminants which result from opening plates to examine them, since it was never necessary to open a plate until the time of subculture. The agar "cut-out" transfer method described first by Klieneberger (66), although not essential for subculturing streptobacillus colonies, gave the best results. The technic has been to cut a rectangular block of agar at the site of good colony growth by using a flattened platinum wire; this agar block, when peeled from the plate by inserting the platinum *spud* under one edge and running it directly beneath, clung to the *spud* and was then transferred to a new plate. The moist colony surface of this *cut-out*, adhering to the agar surface of the new plate, freed the spud which was then used to slide the cut-out along the new agar surface, thereby inoculating a large area. Parafilm was used to seal all inoculated plates as moisture has been found essential to colony growth. Because of the collection of moisture in the lid of the sealed Petri dish following incubation, the dish has been left in the inverted position at all times (incubation, microscopic study, transfer).

In making blood cultures the writers referred to use citrated blood and to concentrate this by centrifugation. Fifteen cc. of whole blood from the patient were introduced into a small flask containing 8–10 cc. of 2.5 per cent sodium citrate. Ten cc. amounts of this citrated blood were centrifuged for 30 minutes in an angle centrifuge at 2500 r.p.m. The supernatant was discarded, and the

blood cell residue cultured by inoculating serum broth tubes with 1 cc. amounts, and the surface of agar plates with several drops. In case 1 the joint fluid was cultured directly on plates and into broth tubes without being citrated and concentrated by centrifugation. In case 3 cultures of the purulent material from the abscess on the sternum were also made directly without concentration. However, in the case of blood cultures it seemed logical to assume that centrifugation would afford the best opportunity for successful culture. The use of 2.5 per cent sodium citrate did not interfere with the growth of *Streptobacillus moniliformis*.

Familiarity with the appearance of the streptobacillus is as important as the correct media. The microörganism grows in the form of *fluff balls* at the bottom of the tube and on the surface of the sedimented blood cells. The supernatant fluid in the tube remains perfectly clear, and a stained smear of this reveals no microörganisms. Unless a portion of one of the balls is withdrawn with a pipette and stained, the evidence of growth will undoubtedly be overlooked. Gram-stained preparations of the balls may lead to further confusion; some show bacterial elements which so closely resemble the blood cellular elements, that recognition of the streptobacillus is very difficult. On the other hand, a Giemsa or Wayson stain of the same material clearly demonstrates the characteristic appearance of the microörganism. Pleomorphism is a striking feature, often suggesting several different organisms and thus the possibility of bacterial contamination.

The use of tubes containing only 5–6 cc. of medium for culturing the blood has been mentioned. The reason for this is that the fluff balls can be easily seen in a small tube, and they may be removed without disturbing the sedimented blood cells. In routine clinical bacteriology blood cultures are almost invariably made by inoculating at least 100 cc. quantities of broth in flasks. It is difficult to avoid disturbing the blood cell sediment in these flasks, thereby obscuring the fluff balls when the attempt is being made to remove them with a pipette. This is another example of the inadequacy of routine methods in isolating the streptobacillus.

Subcultures present another problem. As a general rule, subcultures must be made within 24 hours of the time of inoculation of a tube, since growth of the microörganism causes a drop in the pH of the media to levels of 5.9 to 6.5 in this period of time. Such low pH levels kill the microörganisms if subcultures are not made in time. This is particularly true when using the Dextrose Starch medium. The drop in pH with this medium is definitely lower than with the Tryptose Phosphate (see Fig. 3). We have found that the streptobacillus may not be transferable if one goes beyond the 24-hour limit by even one or two hours. With the Tryptose Phosphate medium in which the pH drop is not so great, the safe period before subculturing may be as long as 36 hours. Only occasionally have subcultures been made at 48-hour intervals with success. A good rule to follow in making the subculture from the original blood cultures, is to make the transfer as soon as possible after the appearance of the fluff balls. As soon as the cultures are growing well some of the material should be preserved by drying or freezing. The writers have found that the microörganisms may be preserved for many months by freezing in a low temperature ice-box (−25°C.). The strep-

tobacillus was recovered without difficulty from some of the original joint fluid from case number one, after having been kept at −25°C. for more than a year and a half. The low temperature ice-box is most useful where unavoidable interruptions in the laboratory routine occur and daily transfers of the cultures are impossible.

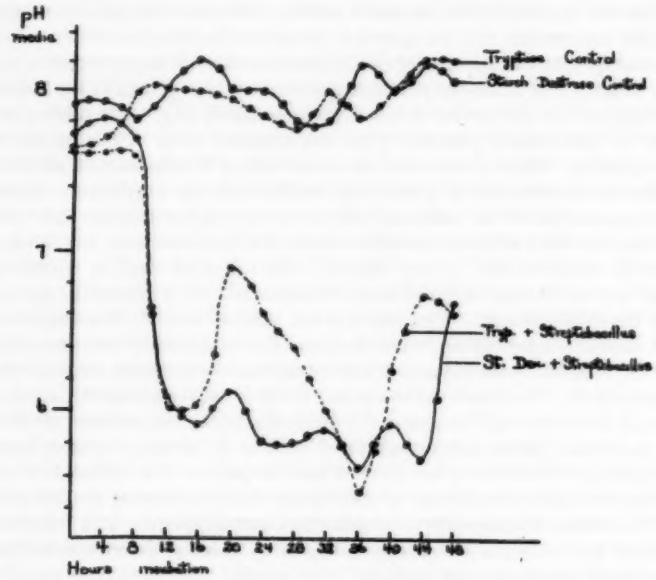

FIG. 3. Curves showing effect of *Streptobacillus moniliformis* growth on pH of Tryptose Phosphate (Tryp), and Dextrose Starch (St. Dex.) broth media enriched with 33 per cent ascitic fluid. Determinations every two hours for 48 hours; glass electrode (Beckman pH meter) method; incubation 37°C. Note sharp drop in pH of both media between 8th and 12th hours of incubation, followed by low sustained pH level of Dextrose Starch media. There is an unexpected final rise in pH of both media between the 40th and 48th hours of incubation. Upper two curves represent uninoculated controls.

Freezing is also important in retaining the virulence of the microörganism. After a number of subcultures the virulence definitely diminishes. For example, the streptobacillus isolated from case 1 became completely avirulent for mice after 400 generations. We were not successful in regaining the virulence by animal passage, once it had been lost. Therefore, if the results of different workers

on lethal effect, production of arthritis, and the effect of chemotherapy, in mice, are to be compared, a clear statement of the number of subcultures undergone by the microörganism should accompany all reports. By freezing newly isolated cultures, one can later obtain at will the same generation for animal pathogenicity studies.

B. Discussion of Bacteriology of Streptobacillus

The typical morphology of this microörganism was well described in the name bestowed upon it by Levaditi *et al.* (80). The name *Streptobacillus moniliformis* indicates chains of bacilli interspersed with beaded swellings. Very excellent morphological descriptions have been given by Litterer, Parker and Hudson, Hazard and Goodkind, Scharles and Seastone, and others. Several features have impressed us.

The streptobacillus is one of the most pleomorphic microörganisms known and this characteristic has led to many conflicting descriptions in the literature. Repeated observations of the various forms it assumes, allows certain very general predictions to be made as to the particular form which will appear under any given cultural conditions; however, totally unexpected forms continue to appear frequently enough to make dogmatic statements hazardous.

The writers are convinced that the microörganism manifests some type of cyclic development; this is influenced by so many variables that only a rudimentary attempt has been made to trace it. Turner (149) has presented evidence favoring a growth cycle in regard to *Pleuropneumonia bovis*.

Speaking only of the streptobacillus and not of the L_1 component, we have first determined upon the basic structure as some sort of *small body*—neither a coccus nor a bacillus. The filament is the next most characteristic structure, and may undergo striking changes. We are not in accord with those who believe the filament is composed only of rigid bacillary units. These filaments vary in size from fine gossamer threads to thick cigar-shaped structures occasionally exhibiting alternate light and dark transverse bands. Large or small spindle-shaped swellings are very common, and have occasionally been seen packed with very faintly staining round bodies (?granules). In the same field one frequently observes transitions in the course of the filament from one to another form and back again. Another striking

characteristic of the filament is its tendency to fragment with age, changes in pH, and other conditions as yet undetermined. This fragmentation is the property which has given rise to the term "Streptobacillus", but it is the exception for the *bacillary* fragments to be uniform in length. Much more common is the irregular *dot-dash* (Morse code) appearance. Along the course of such segmented filaments, one frequently observes swollen, or large round bodies. We are convinced that such bodies are merely swellings in the course of the filaments, and their frequent situation along one side of the axis of the filament is best explained by analogy to the kink in a rope, and best-illustrated in Plate II, Fig. 8.

As will be shown later, these *kinks*, or large round bodies, probably contain the viable elements of the cultures. The large clear *oil-droplet*-like structures thought by Smith to represent *clubs*, which have been commented upon by others, have been the subject of a recent communication by Partridge and Klieneberger (1941) (115), who have indicated the structures' relation to cholesterol. To Dienes, they represent autolysis. We have observed them repeatedly, and feel that they are correlated closely with death of the microörganism. They continue to form even after subcultures can no longer be made. It is felt that they represent merely the swollen "autolyzed" remains of the pleomorphic and bulbous filaments. They are not observed in young, viable cultures. Similar forms are seen in L_1 cultures, and because they so frequently appear below the surface of the agar they tend to be more regularly spindle-shaped, apparently due to peripheral resistance of the medium.

Relatively minor alterations in the media constitute the chief factors in either speeding, slowing, or aborting the development of the supposed streptobacillus cycle. These variations range from the very poorest medium upon which the microörganism will barely survive, to the animal body in which it may again manifest a cycle. We have graded the various media as *poor*, *good*, and *best* according to which supports the most luxuriant and rapid growth. The poorest is the routine stock medium of beef infusion plus Neo-peptone. *Good* is the Tryptose Phosphate, and *best* is the Dextrose Starch broth and agar. All must be enriched with ascitic fluid or animal serum.

Duration of viability of the microörganism is inversely proportional

to the ease and speed with which it grows. Thus, on the best media, although growth is fastest and most abundant, death occurs at least several hours sooner than on the poorer media. This factor influences the morphology considerably. Broth cultures sometimes exhibit extreme pleomorphism and at other times consist principally of filaments and swollen bodies or of tiny filamentary fragments only. This latter situation obtains when the microörganism is *forced*, by rapid transfer in the best medium.

In broth tubes, growth is always at the bottom of the tube and distributed to a variable extent up the side which inclines away from the perpendicular. In poorer media, the gross appearance is that of hard compact balls which may require 48 to 72 hours to appear; as the grade of medium is made progressively *better*, the appearance changes from *hard* to *soft*, fluffy, cotton-like masses occupying $\frac{1}{3}$ to $\frac{1}{2}$ the height of the broth. Finally there is a formed homogeneous sediment which disperses diffusely when the tube is shaken. This latter type of growth always appears early and is maximal in 18 to 24 hours. The *ball* stage is the one most apt to show predominance of round bodies, plus spindles and filamentous fragments of various degrees of pleomorphism. The *cottony* stage is characterized by long, unbroken filaments which extend across many fields and these are either continuous or regularly bacillary in segmentation (Plate II, Fig. 11). In the final homogeneous form, the sediment usually consists of tiny fragments of filaments.

It is always slightly more difficult to obtain growth on solid media than in liquid media. The three rates of growth, *poor*, *good*, and *best*, are obtained on stock, Tryptose Phosphate, and Dextrose Starch agar respectively, as in the case of the corresponding broths. In the preparation of solid media, failure to appreciate the importance of the previously mentioned low percentage of agar and the retention of moisture by plate-sealing (Parafilm), may convert a *best* growth medium into one which will not support growth. In our opinion, two other factors, freshly poured plates and transparency of the media, are also necessary for the best results. These procedures are admittedly time-consuming, but the resultant abundant growth and facilitation of microscopic study of the streptobacillus colonies more than repay the effort expended.

Interesting differences in pleomorphism occur between the microorganisms from colonies on plates and those grown in liquid media. Initial growth is slightly slower on agar than in broth. Growth will frequently succeed in poor broth and fail on the comparable agar medium. Transfer from *poor* broth to *poor* agar frequently fails, whereas transfer from *good* or *best* broth to *poor* agar will often succeed if the attempt is made with a young culture. Transfer from *poor* broth to *better* agar media will succeed. Furthermore, when a broth culture is being transferred at or near the end of its viable period, success more frequently follows if the transfer is made to broth than to agar. By confining our efforts to the three grades of broth and agar, these relationships have become clear after many months of daily transfer.

C. L_1 Pleuropneumonia-like Microörganism of Klieneberger

The formation of L_1 colonies can be microscopically observed within the substance of two to four day old streptobacillus colonies, depending to some extent upon the generation of the broth cultures used to inoculate the plates. The streptobacillus always forms first. With strains passed for only a few generations, the L_1 forms usually appear in the exact center of the streptobacillus colony. As the generations proceed, it becomes apparent that the L_1 colonies develop beneath any part of the streptobacillus colony and in varying number. As Dienes has shown, the L_1 colonies are buried in the agar, and may be obtained merely by washing the streptobacillus off the surface of the plate. More simple than this is Klieneberger's method of waiting for four or more days until the streptobacillus has died and then by the *cut-out* method sliding the piece of agar across the surface of a new plate; this brushes off the non-viable streptobacillus and leaves L_1 colonies growing in the path of the streak or beneath the *cut-out*. As a matter of fact, it is impossible to avoid obtaining L_1 colonies from old streptobacillus strains; attempted plate passage of streptobacillus colonies of the 300th or 400th broth generation invariably results, after a few passages, in a culture of pure L_1 with no streptobacillus elements.

In contrast to Klieneberger's experience, we have not been able to obtain pure L_1 forms directly from broth cultures of the streptobacillus. In innumerable instances, when the streptobacillus has

been allowed to die in the broth, any L_1 components have also died as evidenced by negative plate transfer. The streptobacillus must be grown on plates before L_1 forms can be isolated. Although L_1 colonies regularly develop beneath streptobacillus colonies, they may appear quite by themselves, this tendency becoming more marked as the broth cultures used to inoculate the agar undergo successive passage. The L_1 colonies, in early growth, are microscopic in size but eventually become macroscopic, and appear as minute chalky-white specks which stand out against the greyish-white transluscent streptobacillus colonies. These latter colonies appear very large in comparison and occasionally reach a diameter of 3 mm. Microscopically, the streptobacillus colonies have irregular borders consisting of easily visible filaments with bulbous swellings (Plate I, Fig. 5). On the other hand characteristic L_1 colonies contain round, dark-brown centers which are embedded in the agar and surrounded by delicate lacy peripheral zones, consisting of zoöglia-like material. Occasionally, if the plate is slightly tipped, this surrounding zone may be seen to detach itself and glide across the moist surface of the agar leaving the central mass unchanged. With a few strains of the streptobacillus, we have observed L_1 colonies which consist of only the hard center with very little peripheral extension (Plate I, Fig. 6). Such colonies frequently grow only between two agar surfaces, as first described by Klieneberger. Their viability is much shorter than that of the classic type, and they tend to die after ice-box storage.

Old L_1 colonies have not been adequately described before. They may remain viable for many weeks, and in the course of their slow growth tend to lose the original symmetrical form after about one week. The peripheral extension on the surface of the agar tends to slow down, while the growth of the deeper central mass continues. This central mass becomes more and more irregular as numerous secondary growth centers form. The whole colony becomes much darker and finally after one to three weeks it may present a definite brown to green pigmentation which stains the surrounding agar in halo-fashion. Such old colonies occasionally reach a diameter of 1 mm. (Plate VI, Fig. 23).

There is a third type of colony variation which has drawn little comment in the literature, and which we call Type X. The colony is intermediate in size between the large streptobacillus and smaller L_1 forms and may be described as having a *crumbly* appearance. The

borders are rough and the colony is more coarsely granular than either of the other types. In addition, its color is a slightly deeper brown than that of the streptobacillus, but not as intensely dark-brown as is the center of the L_1 type. Usually filaments along the edge cannot be seen as constantly as in the streptobacillus colonies. These Type X colonies prove to consist largely of the swollen *large round bodies* with less of the fine filamentous growth and more of the flat ribbon-like arrangement seen when the large bodies are numerous. Type X colonies are usually seen when a transfer is made from one grade of broth to a different grade of agar. They may also develop whenever plates are made from broth cultures to which either much less or much more than the optimum amount of serum has been added. We have considered that they represent a step in the direction of the L_1 transformation as a result of growth in slightly inhibitory media. The pure L_1 form only has never been obtained on plates from such broth tubes.

Before going on to a discussion of the microscopic structure of these colonies, it becomes necessary to describe the method of staining which the writers feel has added greatly to their understanding of the supposed *cyclic* changes mentioned above. It is well-known that the Gram method is scarcely applicable to the study of these microörganisms, while that of Giemsa is very good. Much to be preferred to either of these methods is Wayson's stain, a little-publicized stain originally formulated for study of *B. pestis*, and formerly referred to as *Wayson's plague stain* (91). This was suggested to us by Doctor J. Howard Brown and to our knowledge it has not been previously used for the study of the streptobacillus or the pleuropneumonia forms.

Formula (92)

Fuchsin	0.2 gm.
Methylene blue	0.75 gm.
Phenol (99.5 per cent)	10 cc.
Alcohol (absolute)	20 cc.
Distilled water	200 cc.

Directions for preparation

Dissolve the dyes in separate portions of the alcohol, and dissolve the phenol in the water. When dissolved, add the dye to the phenolized water. Filter before storage for later use.

This stain is inexpensive, keeps indefinitely, and has a wide range of uses. It may be used in phagocytic studies, for staining fungi, and for speedy detection of contaminants. A minor objection is its tendency to stain bacilli in bi-polar manner. It requires no dilution, stains intensely in 10–30 seconds, withstands heat to the point of steaming, and gives a beautiful pink and blue differentiation. For example, pneumococcus capsules appear pink, while the bodies of the bacteria stain blue.

In viable cultures, the streptobacillus always stains blue with Wayson's method. The serum elements in the culture medium stain pink, particularly when the stain is rinsed from the slide with water. It is preferable to rinse the slide hastily with 95 per cent ethyl alcohol and immediately to direct a stream of air on to its surface. This procedure dries the film instantly and tends to diminish the amount of stained serum artifact. The staining of the serum elements is much less marked with Giemsa.

For the study of colonies under the oil-immersion lens, we have used the method demonstrated to us and published by Dienes, with the exception that we have used Wayson stain entirely and have not had to shave down the agar block since the medium is entirely transparent. The agar block bearing a colony is cut out and removed from the plate with a platinum spud. It is placed, colony side up, on the center of a slide and allowed to stand under a light for 5 or 10 minutes until the *cut-out* has lost a portion of its excess moisture. To the surface of the agar block is added one loopful of stain, and a cover slip is gently placed upon the stained surface. Melted paraffin is run between the cover slip and the slide to seal and steady the preparation. Intensity of staining may be varied at will by the length of time the stain is allowed to act before application of the cover slip.

Thus, with Wayson stain and by the above technics, the writers have discovered that death of the streptobacillus is correlated with its staining properties. Once the microörganism becomes non-transferable, it loses the quality of staining blue; even though the structure of the pleomorphic elements remains clearly visible, if they stain pink, they are non-viable. As a streptobacillus colony ages a time is reached when only the large round bodies retain the blue stain. However, since subculture is still possible, we believe that these large bodies

serve as *growth-centers*. Many of the bodies appear to contain one or more smaller bodies varying slightly in shape and size. When the method is applied to the Type-X colony, the predominance of loosely packed large round bodies becomes quite apparent.

The same method again applies to L_1 colonies reveals that the central buried mass is composed of tightly packed rounded bodies. If enough pressure is applied to the cover slip to split these colonies, the rounded bodies are seen to be connected with each other by short, poorly-defined filaments; occasionally even a flat ribbon-like form may be demonstrated, identical with those which appear in the course of the unbroken pleomorphic filaments in agar or broth cultures of the streptobacillus. The L_1 colony periphery is disclosed as a delicate zoöglia-like mat which may become ruptured or folded back upon itself when the preparation is made. This periphery is composed of tiny discrete bodies varying in size and shape slightly and separated from each other by a matrix which does not ordinarily take the stain. The vacuoles or autolyzed *droplets* which appear in the peripheries of the old colonies rarely take the stain.

When the L_1 colonies are studied by the method of making simple "impression" preparations directly on a slide and fixing these with methyl alcohol, it is found that the stain fails to disclose the tiny bodies, but rather reveals the interstices of the non-staining matrix so that the periphery takes on the appearance of a reticular network (Plate IV, Fig. 19). This method is wholly inadequate for study of the L_1 colony structure, and has been abandoned, although it is quite suitable for the streptobacillus colonies. It is appropriate to mention here that the appearance of the periphery of these L_1 colonies is similar to that of all other pleuropneumonia-like strains studied. The difference is only one of degree and not of kind. This is one of the reasons for inclusion of the L_1 form with the pleuropneumonia group.

It is our conclusion that the L_1 and the streptobacillus are both stages in the life cycle of the same microörganism and represent neither a symbiosis nor a S-R relationship. We prefer to speak of the L_1 form as a *component*, since the term *variant* implies that the streptobacillus is the chief and essential stage. We are inclined to the belief that the opposite may be true.

The writers like to use the analogy of a plant whose root and stem

structure may remain viable under ordinary conditions, but which flowers into full bloom only under optimal conditions. In this sense, the streptobacillus represents the *flowering* phase in the cycle, while the L_1 represents the plant itself which continues to grow even under moderately adverse circumstances. Whenever the *plant* (L_1) is subjected to optimal conditions, *flowering* (transformation to streptobacillus) invariably occurs. The facts in support of this thesis follow.

Adequate moisture is essential. Liquid medium represents the optimum (protein and carbohydrate concentrations being kept constant), and in our experience the microörganism always grows as a streptobacillus under such conditions.

Very moist sealed agar plates approach this optimum, and the streptobacillus form grows well under such conditions; however, the L_1 form invariably appears in a few days. If the streptobacillus, during the early generations of its passage, is subcultured at one to two day intervals, this form can be maintained. However, a strain which has been carried by daily passage in broth for more than 300 generations cannot be passed by the plate method without becoming converted to the L_1 form after two or three passages. In other words, it has possibly become so adapted to growth in liquid medium that it must revert to the more resistant form in order to live on solid medium.

Whereas the streptobacillus, when growing rapidly under optimal conditions, dies in one to four days in broth or agar, the L_1 form (in the early generations) can live on agar at 37.5°C. in a sealed plate for as long as 50 days and still remain viable on both broth and agar transfer. We have been unable to demonstrate viability after six months under such conditions, even though the L_1 colonies look perfectly normal.

It has proved impossible to pick pure streptobacillus colonies for daily passage on agar in the hope of freeing them from L_1 *contaminants*. In the early generations of any given strain, the streptobacillus colony looks *pure* for the first one or two days. Inevitably after agar passage of such colonies, the L_1 forms begin to appear in five to ten generations.

The possibility of separating the L_1 form from the streptobacillus does not appear to depend upon the generation of subculture of the latter form. The strain of streptobacillus obtained from our first patient was passed in broth for 50 generations, and then the first

L_1 component was isolated on agar. After 232 broth passages of the streptobacillus, a second attempt was made, and again the L_1 form was isolated. After 360 broth passages, a third isolation was successful. Morphologically, there was no difference in the appearance of these three L_1 components. The second L_1 strain isolated was carried on agar plates for a total of 160 passages of which 61 were consecutive single colony passages. Later another 22 passages were made by the same method. The third L_1 strain was passaged for only 30 generations. Their cultural behavior was identical.

We have been able to study the L_1 form only on agar, since invariably, when it was transferred to a broth tube, it reverted to the streptobacillus component in two to four days. A lively controversy has hinged around this point. Dienes, as well as Dawson and Hobby, believe that this reversal phenomenon occurs while Klieneberger, the preeminent authority in this field, denies it. Her thesis is based on the belief that streptobacillus elements are carried hidden in the L_1 *symbiont*, and that prolonged purification of the L_1 colonies by serial passage of single colonies on agar for at least 40 generations is necessary to free the symbiont from its *contaminating* streptobacillus. She criticizes Dienes' assertion on the grounds that insufficient passages were made before the reversal was attempted.

In order to test Klieneberger's beliefs, the writers concentrated on one strain of the microörganism obtained from the first patient. Less extensive observations were made on two other human strains and three rat strains.

The original L_1 component obtained from the first streptobacillus strain was grown on agar for 300 generations. The methods of routine passage were agar block cut-out technic and direct loop transfer. For two periods in the course of passage, single isolated colonies were picked and subcultured for considerable periods of time. The first series of individual colony passages was carried for 121 consecutive generations, while the second was maintained for 40 generations. Whenever agar cut-outs with numerous apparently pure L_1 colonies were incubated in serum broth media, even after 121 agar passages of individually picked L_1 colonies, reversion to the streptobacillus form occurred. On one occasion, the writers thought that the microörganism was growing in broth as a non-reverting L_1 form; small granular balls

formed in the broth which reproduced only L_1 forms on the first subculture plate; continued passage of this granular sediment in liquid media revealed no increased volume of growth, and after eight passages it became clear that the microörganism was dead and non-viable elements were being transferred. Reversion to typical streptobacillus forms in serum broth was always indicated by the development of brush-like or fluff-ball growth extending from the visible L_1 colonies.

It is apparent from the above that Klieneberger's criterion for the proper purification of L's has been satisfied. Never the less, in our experience, reversion has invariably taken place. It occurred to us that our medium (which differs from that employed by Klieneberger) might be more conducive to L_1 reversion. A culture of Klieneberger's non-reverting L_1 organism was obtained from Doctor Dienes, and was tested on our media. The L_1 colonies produced were morphologically similar to our L_1 components and antigenically related to our streptobacillus strains. However, we were not able to make them revert.

Klieneberger's observations are undoubtedly correct, but the present writers question her interpretation. It seems possible that prolonged passage on her medium had so fixed or attentuated the L_1 form that it could no longer revert in liquid media. This contention is further supported by the recent excellent contribution of Heilman (51) who regards the non-reverting L_1 as a *degraded* phase of the *A. muris* (streptobacillus).

We have observed instances of the reversal phenomenon occurring directly on the agar plates, and as the circumstances involved may help to throw more light on the very important symbiont-variant problem we will relate some of these findings.

While attempting to wash L_1 colonies from the surface of a plate with serum broth, it was found that the imbedded central portions remained unchanged although the peripheries were washed away. When the plate was re-inverted, re-sealed, and re-incubated, a drop of serum broth collected in the center of the agar and remained suspended there. Since the entire surface of the plate was seeded with L_1 colonies, the drop of suspended broth in the center was in contact with many colonies. Two days later, the plate was found to contain growing L_1 forms everywhere but in the area containing the drop of fluid, and in that fluid typical streptobacillus was flourishing.

It has been our routine practice to divide the bottom of a Petri dish into three areas with a wax pencil and to pass the L_1 forms from one to the other segment, thus using only one plate for three generations. On at least four separate occasions it was discovered that the L_1 to streptobacillus conversion occurred in the first segment of a freshly made plate. The "purified" L_1 forms appeared on the inoculated first section in 24–48 hours and were then transferred to the next section of the same plate. Several days later, on re-examining the first area, it was discovered that apparent contaminants which had developed with the L_1 colonies were true streptobacillus forms. This happened in each case when the fresh plate was quite moist and when fluid had condensed over the growing L_1 colonies. One one occasion, when horse serum was substituted for the usual ascitic fluid to enrich Dextrose Starch agar, reversal took place. This is an example of a change from a *good* medium to a *better* one, since horse serum always supports better growth than does ascitic fluid.

It was also found that plate reversal could be made to occur by manipulating a surface L_1 colony so that it lay beneath the agar and against the bottom of the Petri dish. This procedure usually forced some fluid out of the Jelly-like medium and into the region between medium and glass. In this situation, typical streptobacillus colonies formed on several occasions.

Finally, a blood culture from our third case of rat-bite fever was made by our usual methods. Typical streptobacillus colonies formed on the surface of the plates. An extra plate was poured by adding 1 cc. of the patient's blood plus 1 cc. of horse serum to 8 cc. of melted Dextrose Starch agar. After incubation for two days, colonies appeared scattered sparsely throughout the entire plate. Those on the surface and bottom of the medium tended to "green" the surrounding agar, and were two to three times as large as the dark colonies scattered through the medium. Subculture of the two types of colonies on to our routine transparent plates then revealed that the surface and the bottom colonies produced typical streptobacillus forms while those buried in the medium propagated themselves as typical L_1 components.

These many observations which have been consistent for a period of two years, have led us to believe that the formation of two or three predictable types of colonies is a manifestation of a developmental

cycle which the streptobacillus shows in response to a host of variable factors. We do not think that the L_1 forms represent sporulation, since this would mean that the spores propagate themselves.

The L_1 form appears to us to be the basic and essential structure upon which the streptobacillus depends for survival. This viewpoint affords a rational explanation of its relation to all the other microorganisms of the pleuropneumonia group. Since pleomorphic filamentous forms constitute one definite type of growth among pleuropneumonia organisms, it seems logical to regard the streptobacillus as a caricature of the other relatives in the family with accentuation of their outstanding features, but yet enough resemblances to afford recognition.

Further evidence in support of this *monistic* theory is afforded by the study of human and animal fluids from which the streptobacillus can be easily cultivated. In the stained joint fluid from our first patient, no microörganisms were seen. A great many smears and sections of affected mouse joints were made, and a variety of staining methods was used, but no ordinary bacteria were ever found. Giemsa-stained preparations of any type of uninfected animal tissue or fluid which contains white blood cells may reveal numerous long filamentous forms, which resembled the streptobacillus. The suggestive forms are no more numerous in known streptobacillus-infected joint fluids than in sterile varieties. We have never been able to identify the the streptobacillus elements as such in purulent material known to be infected with this microörganism. On the other hand, undiluted Giemsa-stained preparations of the episternal abscess material from the third case did reveal many extremely minute unusual elements (Plate III, Fig. 16). The tiny bodies found may represent either the minute forms composing the delicate L_1 periphery, or the very small individual fragments of the filamentous stage of the L_1 component.

It is interesting to revert now to Ogata's observations in which he designated large round and spindle-shaped structures as repreresenting sporozoa, but on later finding filaments, classified the microörganism as an aspergillus. Further study of Proescher's very excellent photographs leads to the conclusion that he was probably dealing with the same tiny bacillus-like bodies which we have found.

D. Streptobacillus Agglutination Reactions

It has been stated by Allbritten *et al.* (1), that it was impossible for them to prepare a streptobacillus antigen suitable for agglutinations. We have also failed to obtain a suitable diffuse antigen when the usual *hard-ball* form of growth has been used. It has seemed unwise to attempt to disrupt physically the filamentous type of growth. When it was found that the streptobacillus could be made to fragment almost completely, our efforts were directed toward obtaining conditions such that the microörganism itself would do the work of producing a form suitable for antigen. This was achieved by using cultures of the 50th to 60th generation which were tending to grow in broth as a homogeneous sediment with uniform dispersal on shaking. One-liter flasks of 20–30 per cent ascitic fluid Tryptose Phosphate broth were inoculated with 5–10 cc. of 18–24 hours' culture. The flasks were allowed to remain in the incubator for three days and were agitated daily. An interesting phenomenon (not previously reported) occurred repeatedly in the large flasks which was never noted in the small tubes. This was the production during the first 24 hours of incubation of a distinct odor suggestive of hydrogen sulfide; this odor disappeared after that period. At first, it was thought that contamination had occurred, but subcultures at this stage were always pure for the streptobacillus. The best antigen prepared by this method came from a flask in which the Tryptose Phosphate medium had been adjusted to pH 7.1 prior to addition of the ascitic fluid.

After three days of incubation, formalin was added in the proportion of 0.125 cc. to 10 cc. of culture and the sediment was centrifuged. The resultant sediment was resuspended in an equal volume of physiologic saline and stored in the ice-box. A diffuse suspension of adequate turbidity for excellent agglutination tests was produced by adding 0.5 cc. of the concentrated antigen to 60 cc. of physiologic saline solution.

Rabbits proved to be highly satisfactory animals for immunization studies. Intravenous injections of sediment from 5 cc. broth cultures enriched with normal rabbit serum (10–30 per cent) were given at two to three day intervals for 10 to 14 days. The agglutinin titres reached 1/1280 repeatedly, and 1/10,240 occasionally. Routine Wassermann tubes were used; after vigorous shaking, the

tubes were incubated in a water-bath at 52°C. over-night, and read either immediately the next morning or preferably after being chilled for several hours. This method eliminated the proagglutinoid zone (in the first three tubes) which usually resulted from incubation in the 37.5°C. water-bath for two hours followed by over-night chilling.

The three human and the three rat strains studied in this laboratory were agglutinated to practically the same titre degree with the sera from rabbits immunized against the first human and first rat strain studied. No immune sera were prepared against the remaining four strains.

Rabbits were immunized with virulent as well as with attenuated avirulent forms of the same strain. Agglutinin production was proportional to virulence. Although living cultures of the streptobacillus were always used for immunization, no disease was noted in the rabbits other than occasional slight transient weight loss. Agglutinins usually disappeared completely after three months, but returned to a level of at least 1/1280 after several reinjections of antigen.

There was cross-agglutination with only two of a number of strains of pleuropneumonia-like microörganisms studied. One strain isolated from a normal mouse conjunctiva (CFI strain of mice) produced a toxin which, when sterilized by Seitz filtration, killed mice within 24 hours when given intravenously in doses as small as 0.1 cc. Rabbits produced good agglutinins against this microörganism to a titre of 1/640. However, the same rabbit immune sera agglutinated three of the streptobacillus strains in dilutions of 1/160 to 1/320. It agglutinated one rat strain of streptobacillus only in a dilution of 1/10. Two other human strains were not tested. Rabbit sera immune to one human strain and one rat strain of streptobacillus failed to agglutinate the mouse pleuropneumonia microörganism.

The writers have worked with five strains of pure pleuropneumonia-like microörganisms obtained from rat lungs, all of which were morphologically identical. These strains do not resemble L_1, and probably correspond to Klieneberger's L_3 (67). The serum of a rabbit immunized against the first of these rat microörganisms showed agglutinins in a dilution of 1/640. This same serum showed agglutinins against the other four strains in dilutions of 1/160, 1/20, 1/5120,

and 1/160 respectively, apparently indicating some serologic difference in spite of the morphologic identity of the four strains.

Serum from a rabbit immunized against the rat pleuropneumonia strain (not L_1) agglutinated the first human streptobacillus in a dilution of 1/160. Streptobacillus immune serum was found to agglutinate one rat pleuropneumonia strain in a dilution of 1/160. No rat pleuropneumonia strain (similar to those described above) has ever been isolated from a streptobacillus form. This would seem to indicate a serologic relationship which does not correlate with morphologic or cultural properties, since the rat pleuropneumonia strains and the L_1 strains bear only a superficial morphological resemblance. On the other hand, sera from four patients with rat-bite fever, all of which showed agglutinin titres against the streptobacillus of 1/320 or more, failed to agglutinate the rat pleuropneumonia forms; three such sera failed completely when tested against the mouse type of pleuropneumonia-like microörganism.

It is difficult to explain all of these relationships, but the results seem to indicate that certain animal pleuropneumonia-like microorganisms have a greater potentiality for calling out agglutinins against the streptobacillus than has the streptobacillus for stimulating the production of agglutinins against the animal L forms. Also, whereas animals immunized against the streptobacillus may develop agglutinins for animal strains of pleuropneumonia-like microörganisms, human serum of equal titre against the streptobacillus fails to contain agglutinins for these animal pleuropneumonia forms.

Sera from patients with rat-bite fever have repeatedly been tested against the various animal strains of pleuropneumonia-like microorganisms, but specific agglutinins for these L forms have never been demonstrated.

Mice with experimental streptobacillus arthritis developed specific agglutinins against this microörganism. In one instance, three mice were injected. Two developed arthritis, and one remained healthy. Serum of the healthy mouse contained no agglutinins while the sera from both of the arthritic mice revealed streptobacillus agglutinin titres of 1/320. One of the two arthritic mice was treated with gold after development of symptoms. This therapy did not appear to alter the agglutinin titre.

An attempt has been made to rule out the possibility of cross-anti-

genicity between *Spirillum minus* and *Streptobacillus moniliformis*. Pooled sera from mice heavily infected with *Spirillum minus* were tested for the presence of streptobacillus agglutinins with negative results. Sera from *Spirillum minus* infected guinea pigs are being investigated at the present time, and so far there has been no indication of the presence of streptobacillus agglutinins in any of these sera. These findings would appear to support the specific rôle of the streptobacillus in cases 4 through 8 of our series. Reproducible agglutinin titres were demonstrated repeatedly in our patients. The levels obtained have been previously referred to, and summarized in Tables V and VI.

In order to test the specificity of the streptobacillus agglutination reaction in rat-bite fever, we have investigated a number of control human sera—samples from 50 healthy blood donors, and 50 hospital patients suffering from a variety of medical, surgical, and obstetrical conditions, acute infections, blood dyscrasias, neoplasms, hemorrhages, unexplained arthritis, etc. In addition, 20 sera were obtained from patients with positive serologic tests for syphilis. In no instance did the agglutinin titres of these control sera exceed 1/40 when tested against *Streptobacillus moniliformis*. Agglutination was almost invariable in a dilution of 1/10, and quite frequent in 1/20 and 1/40. We have concluded, therefore, that a streptobacillus agglutinin titre of 1/80 or above is indicative of infection with this microörganism.

The presence of significantly elevated agglutinin titres against the streptobacillus in all of the rat-bite fever patients has been demonstrated repeatedly with antigen checked by normal and hyperimmune rabbit serum. Certain conclusions can be drawn from an analysis of Tables V and VI (summary of agglutinations), particularly in the cases where samples of serum were obtained throughout the course of the acute illness and following recovery: (1) Specific streptobacillus agglutinins may make their appearance as early as 10 days after the bite (case 2), and as late as 2½ months (case 8); (2) the maximum agglutinin level may be expected between one and three months after the bite; (3) agglutinins may disappear as early as five months after infection (case 2), or persist in high titre for at least 2½ years (case 5), and in low titre for 7 years (case 4).

ANIMAL PATHOGENICITY OF STREPTOBACILLUS MONILIFORMIS

Streptobacillus infection occurring as a fatal epizoötic disease in colonies of mice was reported by Levaditi, *et al.* (81) in 1932 and Mackie, *et al.* (85) in 1933. Some years before these reports had appeared (1917), Tyzzer (150) described a fatal epizoötic disease in a colony of Japanese waltzing mice. This disease may have been due to the streptobacillus although the pathological findings in the mice (chiefly enteritis and focal necrosis in the liver) were somewhat different from those described by Levaditi and Mackie.

Levaditi drew a striking parallel between this spontaneous disease in mice and acute rheumatic fever in man, pointing out that the endocardium, myocardium, pericardium and joints were the tissues chiefly affected in both disease entities.

The present writers have not observed spontaneous streptobacillus infection in mice. (We have isolated the streptobacillus from purulent middle-ear disease in rats, as well as from normal rat lungs. Recently, we have recovered a pure culture of L microörganisms, morphologically distinct from L_1, from the joint of a rat with spontaneous arthritis. Many of the features seen in the epizoötic disease in mice have been reproduced in experimentally induced streptobacillus infection.

All of the streptobacillus strains isolated by the writers were virulent for mice. The type and severity of infection depended upon two main variables, the generation of the culture used, and the particular strain of mice inoculated. The streptobacillus lost its virulence completely after 400 generations, and passage through mice failed to restore it. CFW[6], a virus-susceptible strain of mice, developed a far more severe and rapidly fatal infection than CFI, a virus-resistant strain. Recognition of the variable factors has made it possible to produce at will either an overwhelming septicemia and death within 24 hours (in which case the effect of chemotherapy could be tested to best advantage), or a chronic, low grade infection with arthritis as the chief manifestation. Infection was produced by intravenous, as well as by intraperitoneal inoculations with 16 to 20 hour cultures of the microörganism. We have employed the intravenous route

[6] Mice obtained from Carworth Farms, New City, New York.

most often, injecting 0.1 to 0.5 cc. of broth culture. Intraperitoneal infection usually required 0.5 cc. of inoculum.

A. Chronic Infection in Mice

In the chronic type of infection (CFI mice, about the 50th generation of streptobacillus) the following findings were noted:—

Arthritis. This was the most striking feature of the experimental infection. (No other human pathogenic microörganism known produces arthritis so regularly in any experimental animal following intravenous or intraperitoneal inoculation). Plate X, Fig. 39 is a diagrammatic representation of the migratory character of the arthritis in the early stage (first three weeks). During this period blood cultures were irregularly positive. No positive blood cultures were ever obtained after three weeks, and from this time on the arthritis became localized in one or more joints. The ankle and wrist were most frequently affected although at times the toes were also involved. Fusiform swelling of the digits provided a striking picture (Plate VII, Figs. 25 and 26). Arthritic changes in the region of the intervertebral discs of the spine and tail sometimes occurred. The pathological picture was chiefly one of marked destruction and necrosis, with some general proliferative changes, and thickening of the joint capsule (Plates VIII and IX). Following the initial stage of the infection, which tended to be migratory, the joint fluid became thick and caseous with a predominance of polymorphonuclear cells. We have never noticed spontaneous suppuration of any affected joint, nor have we observed healing of the arthritic process, once it has been firmly established following the early migratory phase. Furthermore, we have never failed to recover the streptobacillus by culture even as late as 80 days (only a few mice were carried this long), after the onset of the infection. In spite of the ease with which the streptobacillus could be cultured, innumerable Giemsa and Wayson stained preparations of the purulent joint exudate invariably failed to reveal any recognizable streptobacillus elements. We have referred to this point previously, and we feel that it is very important for two reasons: (1) Without the use of special streptobacillus culture methods the arthritis would be considered sterile. (2) The streptobacillus has appeared to exist in morphologically obscure L form in infected joint tissues.

Anemia. Marked pallor of the ears and tail was noted in about 25 per cent of the chronically infected mice. In a few instances where erythrocyte counts were made, a reduction of about 50 per cent of the normal was observed. In spite of this anemia there was no apparent weakness nor oedema.

Diarrhea. This occurred in about 25 per cent of the mice, and was almost always accompanied by emaciation, general debility, and uncleanliness. The stools were not cultured for the streptobacillus; blood cultures when positive revealed only this microörganism. We have attempted to infect mice by the gastrointestinal route (stomach tube). Neither diarrhea nor any other signs of infection resulted. Diarrhea was noted only following intravenous inoculation, although other routes of inoculation were less frequently employed.

Conjuctivitis. Following the use of a virulent strain of the streptobacillus, conjunctivitis was likely to occur. Both eyes were usually involved, and it frequently cleared spontaneously.

Paralysis. A. *Transient.* A flaccid paralysis, almost always unilateral, involving a hind limb, was occasionally observed. The leg was fully extended and was dragged around. The paralysis usually lasted for only one or two days, then cleared spontaneously, and the leg became useful again.

B. *Permanent.* Both hind legs were invariably involved in this instance, and in addition kyphosis and usually priapism were present. The penis became dry, emaciation developed and death occurred in one to two weeks. At autopsy the bladder was greatly distended. No convulsions were seen. Levaditi mentioned the kyphosis of the spine with resultant pressure on the spinal cord and paralysis.

Alopecia. Loss of hair occurred frequently about the eyes and noses of mice with longstanding infections, but it was difficult to associate it with the presence of the streptobacillus. In this connection, it is of interest that Mooser (98) (99) described similar alopecia around the eyes of *Spirillum minus* infected guinea pigs, and he considered this an important diagnostic sign.

We have not observed any changes in the endocardium, myocardium or pericardium similar to those described by Levaditi. Occasionally small white spots were seen in the liver.

B. Prevention of Infection with Gold (Sodium aurothiomalate) Therapy

As reported by Heilman (50) and the writers (13), gold possesses a potent chemotherapeutic action in the prevention of infection in mice, whereas sulfonamide drugs have no effect. Gold has been found to be remarkably effective in the prevention of experimental infection with members of the pleuropneumonia group in general (32) (126). We have observed that the most susceptible mice (strain CFW) infected with a highly virulent freshly isolated strain of streptobacillus, were protected completely with 1 mgm. (subcutaneously) of sodium aurothiomalate (Myochrysine) and none of the complications mentioned above occurred. Furthermore, repeated blood cultures in this treated group remained sterile, whereas in the controls and sulfonamide (sulfanilamide, sulfapyradine, sulfathiazole) treated mice the blood cultures remained positive for the streptobacillus until death occurred in 24–48 hours; in the case of a few mice arthritis developed and when this occurred life was almost always prolonged for a week or 10 days, the blood cultures remaining positive during this period. When a less virulent culture of streptobacillus (about the 50th generation) was used to infect the more resistant CFI strain of mice and the chronic arthritis developed, the daily subcutaneous administration of myochrysine for a period of two weeks had no effect. The streptobacillus was recovered from the affected joints following such treatment.

It is of interest that Heilman (50) has shown the failure of arsenicals, unlike gold, to protect mice against streptobacillus infection. A slight protective action of some of the arsenicals has been demonstrated by Findlay, *et al.* (32) in mouse infection with certain strains of pleuropneumonia-like microörganisms (L_6, L_8, and L_7, but not L_4).

NOMENCLATURE AND CLASSIFICATION

We have retained the term *Streptobacillus moniliformis* because of its descriptive value and because the majority of the bacteriological literature uses this term. It definitely antedates *Haverhillia multiformis* and connotes more of morphological significance. The chief morphological distinction which the writers make from many of the previous descriptions, regards branching. We do not believe we

have yet demonstrated true branching. Frequently, false branching occurs because of the apposition of fragments from filaments, but that is all. We suggest the term L_1 *component* for the L form always associated with the streptobacillus. The term *component* avoids the symbiont-variant controversy which has not been generally settled.

For the purpose of more exact classification the writers are inclined to accept as least ambiguous and least confusing the name *Actinomyces muris* as suggested by Topley and Wilson (145). We have become more so inclined lately after having studied a strain of *Actinobacillus actinomycetem-comitans* obtained by us in pure culture from a patient known to have chronic actinomycosis of bones, lungs, and skin. We have been unable to demonstrate "reversal" to *Actinomyces bovis* with this culture, but in other respects, it closely mimics the behavior of L_1 forms. The existence of two quite distinct morphological forms in an accepted member of the actinomyces group provides additional grounds for including the streptobacillus under the same genus, using the species name *muris* for specific identity.

CONCLUSIONS

Evidence of the etiological rôle of *Streptobacillus moniliformis* in eight cases of rat-bite fever has been presented. This evidence has been based on the clinical picture, the successful recovery of the microörganism from three cases (blood of all three, joint fluid of one, abscess of one), and the finding of specific streptobacillus agglutinins in all of the eight cases. The specificity and value of the streptobacillus agglutination reactions has been demonstrated by testing 120 control human sera, and the sera from mice and guinea pigs infected with *Spirillum minus*. Several of our cases from which numerous samples of serum were obtained during and following the entire febrile illness, revealed an initial rise and a subsequent fall in the agglutinin titres. This provided clear-cut evidence of the infectious rôle of the streptobacillus. In one of the cases streptobacillus agglutinins were present six years after the acute illness. Unsuccessful attempts to demonstrate coexisting *Spirillum minus* infection were made in four of the eight cases (animal inoculation in three and direct examination in only one).

Each of the three strains of streptobacillus recovered from the patients and two additional strains isolated from rats possessed

marked virulence for mice. A gold preparation (sodium aurothiomalate) protected mice against streptobacillus infection, whereas sulfonamide drugs exerted no protective action.

The L_1 pleuropneumonia-like microörganism of Klieneberger was isolated from the five strains of streptobacillus which we have studied. The L_1 form always reverted to the streptobacillus under appropriate culture conditions, even after 121 consecutive passages on agar of individually picked L_1 colonies (purification method suggested by Klieneberger). Because of this consistent reversion phenomenon, and other experimental evidence which has been discussed fully, we have concluded that the L_1-streptobacillus relationship is one of bacterial variation, and not symbiosis. Repeated observations have been cited to indicate that the streptobacillus usually exists in the morphologically obscure L_1 form in infected human and animal tissues.

From a review of the entire subject of rat-bite fever, there is good evidence that the *Spirillum minus* causes an illness which may be indistinguishable from that produced by the streptobacillus. The implication has been made that some cases may have been infected with both agents. The clarification of the relative etiological significance of the two microörganisms in rat-bite fever will be furthered by the proper search for both agents in future cases.

Evidence has been presented which suggests that *Streptobacillus moniliformis* infection is induced with greater frequency by the bite of a rat than is *Spirillum minus* infection.

In the year 1941, although not included in the period covered by this analysis, two cases of rat-bite fever have been reported in which the spirillum was obtained by animal inoculation (Packchanian and Sweet (112), and Greengard and Hess (45)). This second patient (45) died, but it seems unfair to accept this infection as due to the *Spirochaeta morsus muris* because in the entire article the words *streptobacillus* or *streptothrix*, are not mentioned, blood cultures were "consistently negative for spirochetes", no smears nor bacteriological studies were reported on the autopsy material, and the patient was treated with anti-rabies vaccine but not with arsenic. The infant died of an "intercurrent infection with hemorrhagic bronchopneumonia" upon which bacteriologic studies were not reported, but for which sulfapyridine was given. In the light of present knowledge, the writers question the etiology of the bronchopneumonia, the rationale for the anti-rabies treatment, and the wisdom of withholding arsenic.

Recently Larson (see footnote, page 267 for reference) reported three cases of

rat-bite fever. *Spirillum minus* was obtained from case 1 by animal inoculation, the streptobacillus was recovered by culture from case 2, and streptobacillus etiology was considered proven in case 3 by agglutination methods only.

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PLATE I

FIG. 4. Colonies of *Streptobacillus moniliformis* recovered from Case 1. Grown on 20 per cent ascitic fluid beef infusion agar; incubation 48 hours, 37°C. Indirect lighting showing raised granular appearance of colonies. × 70.

FIG. 5. Colonies of *Streptobacillus moniliformis* recovered from Case 2. Grown on 20 per cent ascitic fluid Dextrose Starch agar; 9th generation, incubation 7 days, 37°C. Direct lighting showing lacy filamentous structure. The swollen bodies along the course of the filaments are barely visible at this magnification. × 70

FIG. 6. Colonies of the *L_1 microörganism of the pleuropneumonia group* separated from the *Streptobacillus moniliformis* strain recovered from Case 2. Grown on 20 per cent ascitic fluid Dextrose Starch agar; 6th generation, incubation 48 hours, 37°C. This illustrates the appearance of L_1 colonies which have not yet developed the characteristic finely granular peripheral zones noted in Fig. 7. × 70

FIG. 7. Colonies of the *L_1 microörganism of the pleuropneumonia group* separated from the *Streptobacillus moniliformis* strain recovered from Case 1. Grown on 20 per cent horse serum Dextrose Starch agar. 64th generation, incubation 48 hours, 37°C. This is the appearance of L_1 colonies grown under ideal conditions. The largest colony of the group shows the unusual development of a secondary growth ring. × 70

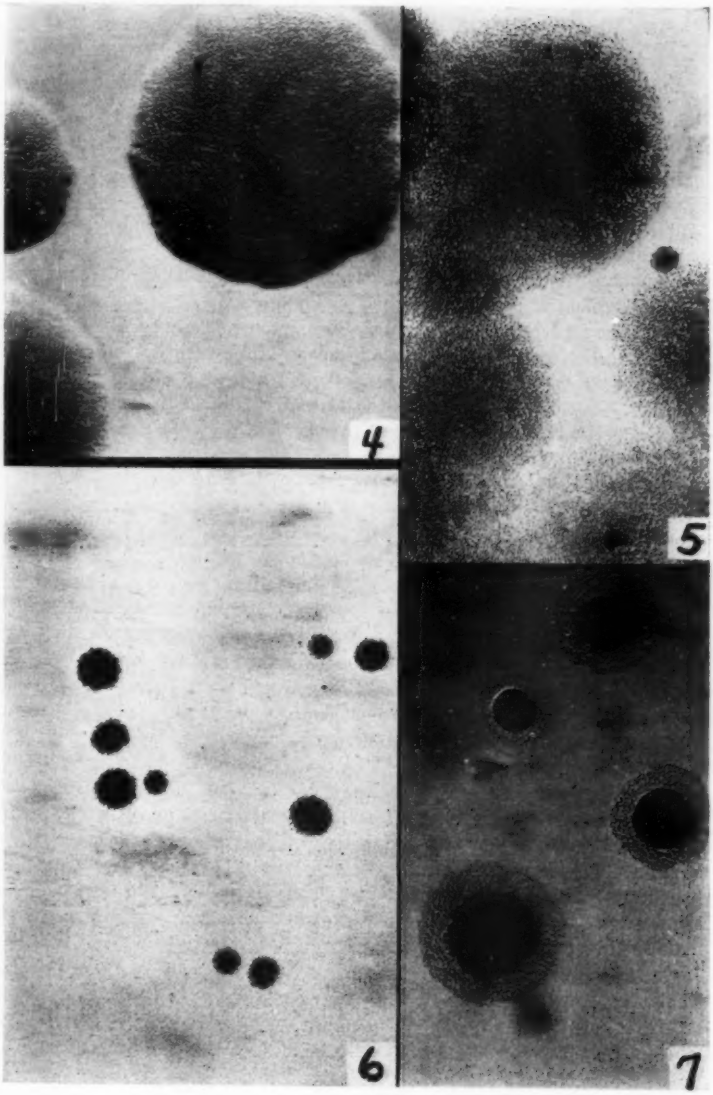
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PLATE II

FIG. 8. *Streptobacillus moniliformis.* Impression preparation from colonies grown on 20 per cent ascitic fluid beef infusion agar; incubation 72 hours, 37°C. Wayson stain (alcohol washed). Here the filaments present a segmented, beaded appearance. The preparation also appears to show the way by which the large, swollen, eccentrically located bodies develop. The large bodies seem to be composed of smaller swollen units which originally develop in groups along the course of otherwise fairly uniform filaments. These groups finally appear to be drawn tightly together making a rounded thickened kink which protrudes as a solid mass from the side of a filament. × 1000

FIG. 9. *Streptobacillus moniliformis.* Culture conditions similar to those noted under Fig. 8, with Giemsa replacing Wayson stain. The filaments stained with Giemsa generally appear more delicate and do not present the degree of segmentation and beading seen with the Wayson stain. Two types of swollen bodies are to be noted along the course of the filaments; the elongated, symmetrically placed, spindle-shaped forms, and the rounded globular bodies (described in Fig. 8) which protrude from the side of the filaments. × 1000

FIG. 10. *Streptobacillus moniliformis.* Impression preparation of a colony. Grown on 20 per cent ascitic fluid beef infusion agar; incubation 48 hours, 37°C. Wayson stain (water washed). This demonstrates how a slight variation in the staining technic may alter greatly the appearance of the streptobacillus. Here the large swollen bodies as well as the small segmented elements making up the filaments, present a partitioned, foamy, vacuolated appearance. × 1000

FIG. 11. *Streptobacillus moniliformis.* Preparation from liquid media. 111th generation of strain recovered from Case 1 grown in 33 per cent ascitic fluid Tryptose Phosphate broth; incubation 24 hours, 37°C. Wayson stain (alcohol washed). Because of ideal culture conditions, as well as the rapid transfer method, the sedimented microörganism growth used for this preparation presented a stringy, diffuse, mucoid appearance rather than the usual characteristic fluff ball streptobacillus aggregates. Under such circumstances the globular and spindle-shaped swellings may be absent, as in this preparation. Note the twisted mass of fairly uniform filaments. × 1000

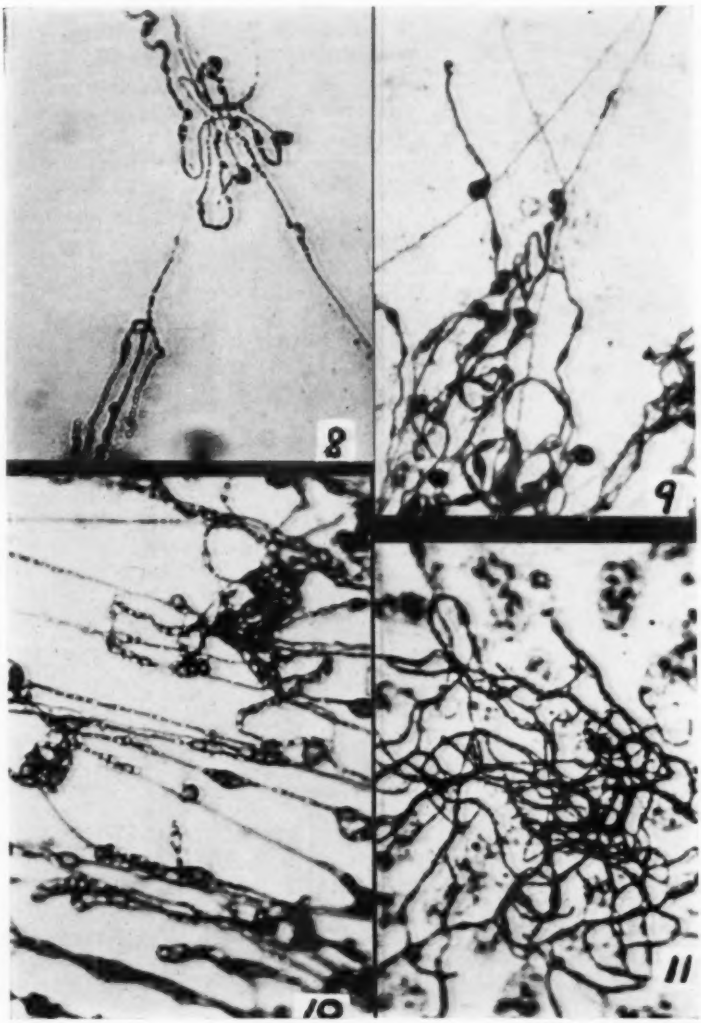
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PLATE III

FIG. 12. *Streptobacillus moniliformis.* Grown in 33 per cent ascitic fluid Tryptose Phosphate broth; incubation 24 hours, 37°C. Wayson stain (alcohol washed). Culture represents 2nd subculture following reversion of L_1 microörganism to the streptobacillus form. Note large spindle-shaped form with the delicate thread-like filaments. × 1000

FIG. 13. *Streptobacillus moniliformis.* Grown in liquid media similar to that described in Fig. 12; incubation 24 hours, 37°C. Wayson stain (alcohol washed). 2nd subculture following reversion of L_1 microörganism to the streptobacillus. Note the extreme degree of pleomorphism. The large snake-like form is unique. × 1000

FIG. 14. *Streptobacillus moniliformis.* Grown in 33 per cent ascitic fluid beef infusion broth; incubation 48 hours, 37°C. All of the forms seen vary somewhat in length and are bacillary in type. The preparation represents a frequently encountered form of the streptobacillus which, because of the absence of spindle-shaped or globular swelling, does not necessarily suggest the streptobacillus picture. The bacillary elements result from the breaking up of filaments. × 1000

FIG. 15. Impression preparation of the edge of a *Streptobacillus moniliformis* colony showing the twisted filamentous character of the growth. × 450

FIG. 16. A Giemsa stained preparation of pus from the abscess in Case 3. A pure culture of *Streptobacillus moniliformis* was grown from this pus. High magnification, (× 1500) was necessary to demonstrate very small forms. No filaments or spindle-shaped elements or large swollen bodies were ever seen in repeated examinations of this pus. At the usual oil immersion magnification of × 1000 these elements more closely resemble pleuropneumonia forms seen in Fig. 17. × 1500

FIG. 17. *L_1 microörganism* obtained from Dr. Dienes who in turn received it from Dr. Klieneberger in England. This strain was originally separated from a culture of *Streptobacillus moniliformis* by Dr. Klieneberger and unlike our strains of L_1 microörganism, has never been known to revert to the streptobacillus form. This preparation was made from a 4-day culture grown in 10 per cent horse serum Brewer's thioglycollate anaerobic medium. One of the ball-like aggregates was stained in the non-fixed moist state with Wayson stain and mounted beneath cover slip. Note the complete abscence of streptobacillus elements. Under these conditions of growth and staining the L_1 microörganism has appearance of an amorphous mass of varying sized particles some of which are small straight and curved bacillary forms. Note also the oily droplets which Klieneberger has described in conjunction with L_1 growth. × 1000

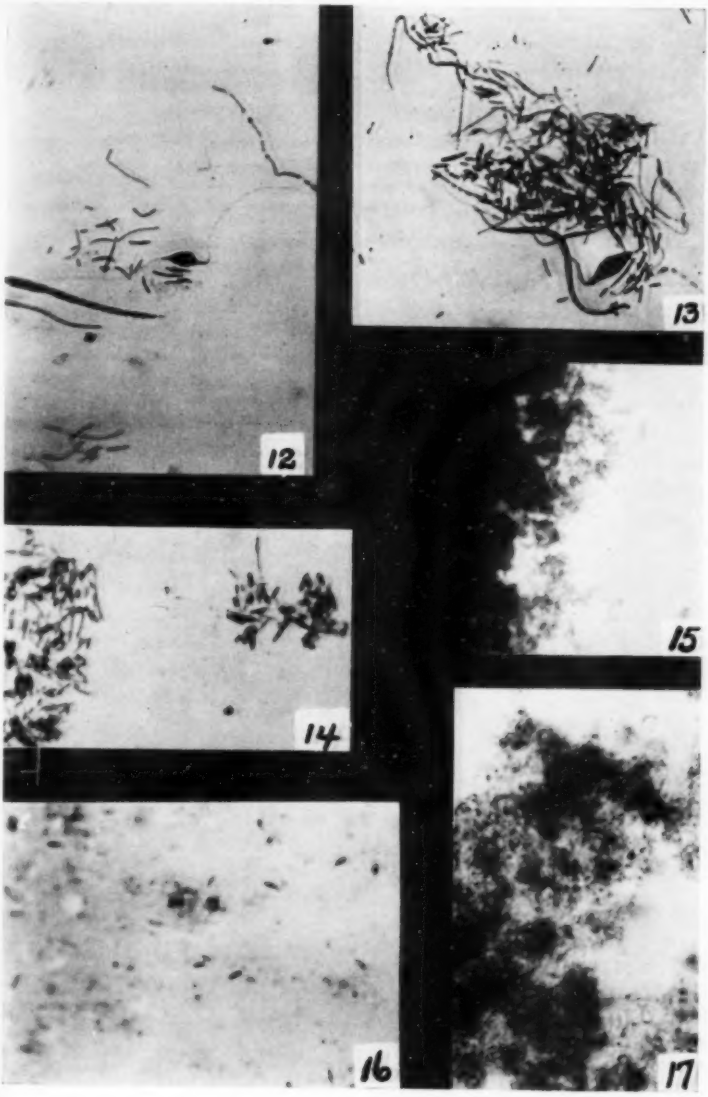
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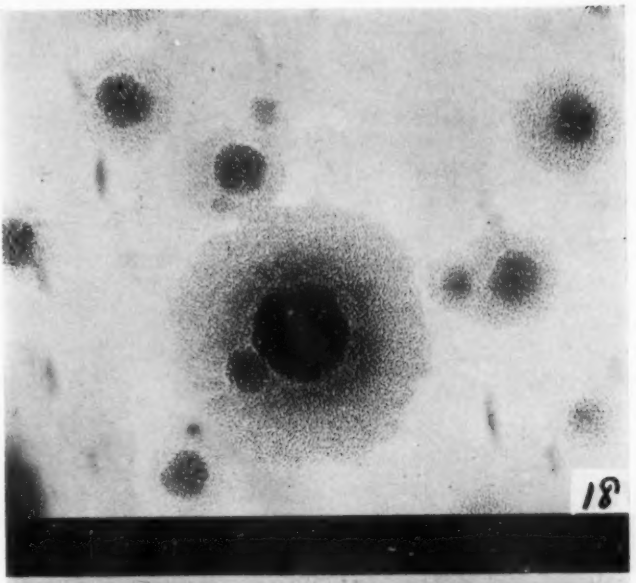
PLATE IV

FIG. 18. Colonies of *L_1 microörganism*. Klieneberger's non-reverting strain originally separated from *Streptobacillus moniliformis*. Grown on 20 per cent horse serum Tryptose Phosphate media; incubation 4 days, 37°C. Note the "double nucleated" appearance of the large colony. × 100

FIG. 19. Wayson stained impression preparation (fixed absolute methyl alcohol) of *L_1 colony* isolated from streptobacillus (strain from Case 1). Colony grown on 33 per cent ascitic fluid stock (beef infusion) agar; incubation 48 hours. The reticulated upper portion corresponds to the peripheral zone of the L_1 colony, and the dark area represents the center. Note the absence of ordinary bacterial elements. × 1000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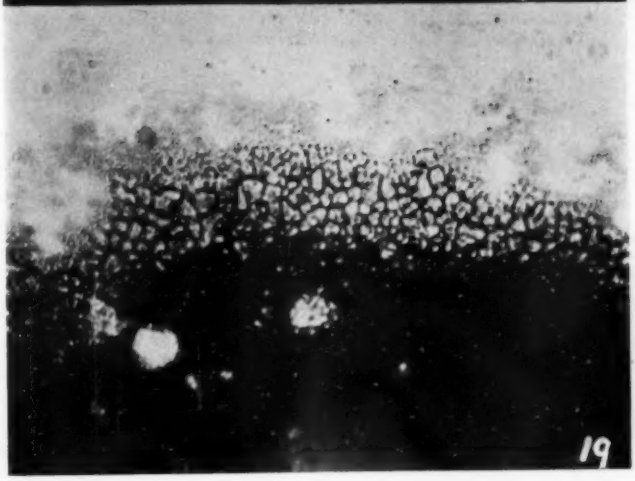
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PLATE V

FIG. 20. Colonies of *Actinobacillus actinomycetem-comitans*. Isolated in pure culture from a case of actinomycosis by J. C. N. Grown aerobically on 20 per cent horse serum Dextrose Starch agar; incubation 48 hours, 37°C. These colonies were only visible by microscopic examination of the agar plates. Note the hard, waxy, refractile character of the colonies. These microörganisms bear much the same relationship to Actinomyces bovis, as does the L_1 to the streptobacillus. × 100

FIG. 21. Colonies of *Actinobacillus actinomycetem-comitans*. Stained on the agar with Wayson stain. Grown on 20 per cent horse serum Dextrose Starch agar; incubation 2 months, 37°C. Old colonies such as these may reach 0.1 to 0.5 mm. in diameter, indicating the generally minute nature of these colonies. × 320.

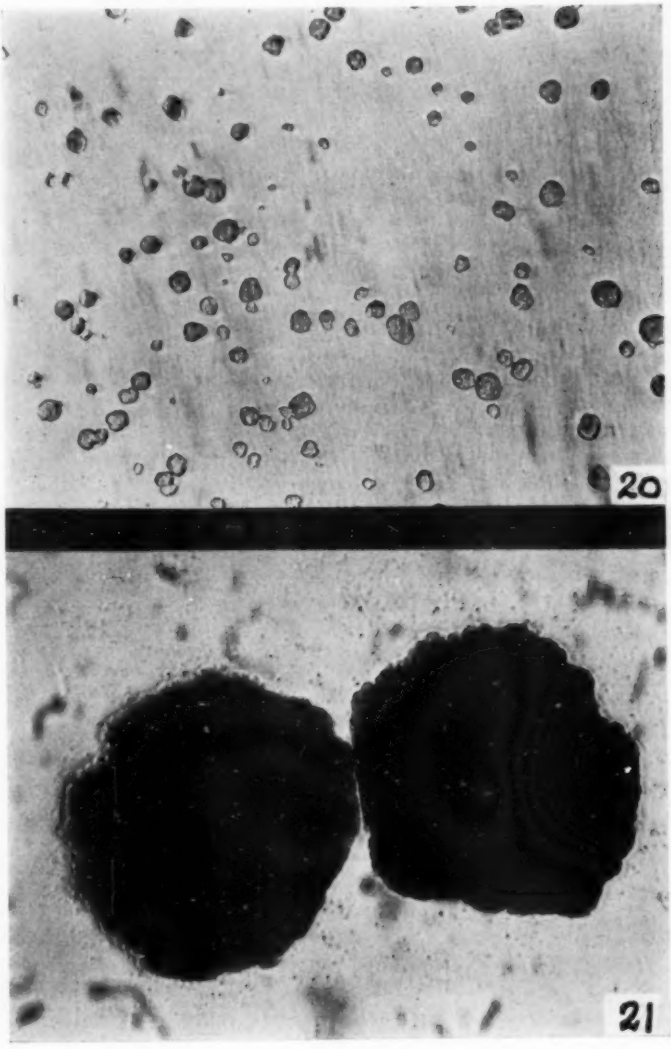
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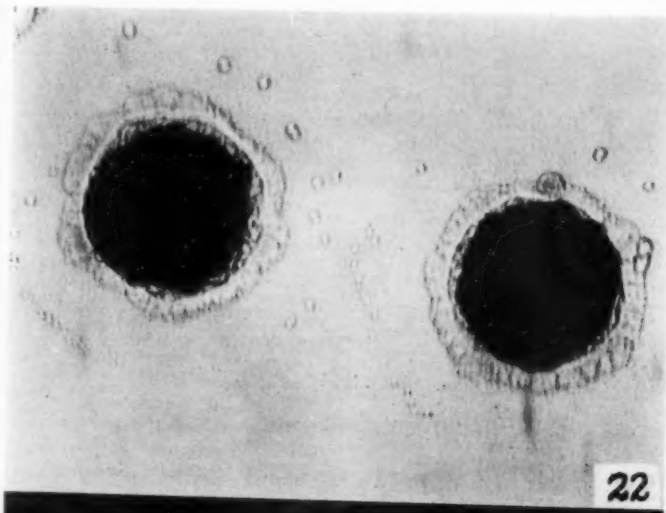
PLATE VI

FIG. 22. Colonies of *Actinobacillus actinomycetem-comitans*. Grown aerobically on 20 per cent horse serum Dextrose Starch agar; incubation 14 days, 37°C. This particularly preparation shows some similarity to the L_1 microörganism (dark center and light peripheral zone). The usual appearance (Fig. 20) bears little morphological similarity to L_1 except for the small size of the colonies. × 320

FIG. 23. Colonies of *L_1 microörganism*. Strain separated from streptobacillus from Case 1. Grown on 20 per cent horse serum Dextrose Starch agar; incubation 14 days, 37°C. Note the large dark colony. This appearance of the L_1 colonies is frequently encountered in old cultures of this microörganism. Although not clearly seen here the central portion of this type of L_1 colony often presents a waxy, refractile, "hard" appearance which resembles old *Actinobacillus actinomycetem-comitans* colonies. × 320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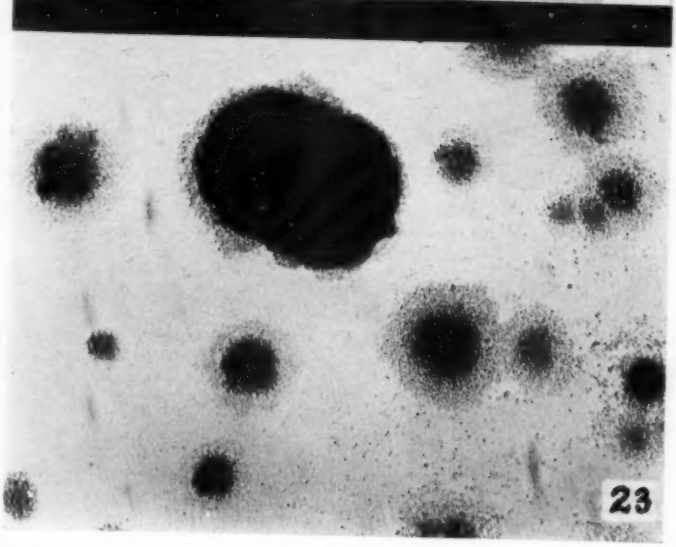
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PLATE VII

FIG. 24–29. Experimental arthritis in mice produced by intravenous injection of *Streptobacillus moniliformis*. Note the involvement of the tail, ankle, toes, and wrist joints.

FIGS. 30–31. X-rays of affected ankle joint and tail, showing the severe destructive changes as well as the soft tissue swellings produced by *Streptobacillus moniliformis* following intravenous injection of the microörganism.

FIGS. 32–35. Unaffected control mice showing normal joints to be compared to the affected ones above.

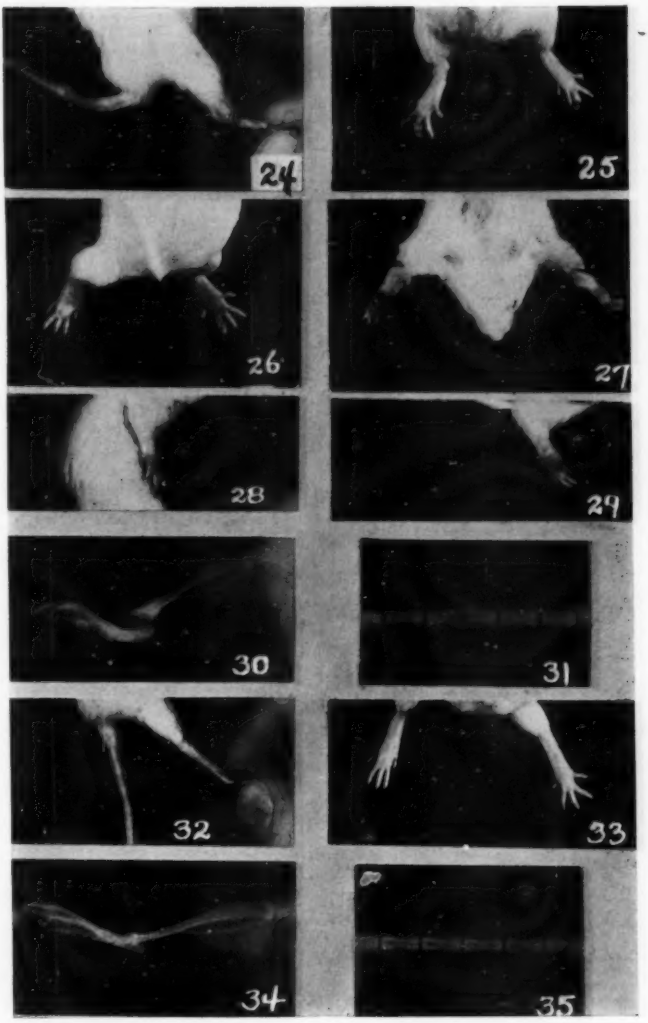
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PLATE VIII

FIG. 36. Affected wrist joint of mouse showing irregular appearance due to the destruction of articulating cartilage, replacement with cells, and proliferation of tissue into the joint space. In this instance the arthritis followed intraperitoneal injection of *Streptobacillus moniliformis*. H & E stain. × 65

FIG. 37. Normal wrist joint of mouse for comparison with affected joint shown above. H & E stain. × 65

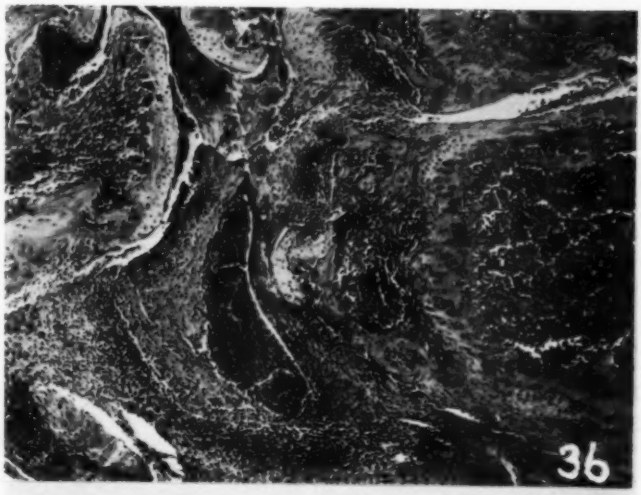
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PLATE IX

FIG. 38. Tail of mouse showing the marked changes due to streptobacillus infection. Note the obliteration of the intervertebral disc and the epiphyseal lines on either side, as well as the extension of the cellular and proliferative reaction into the subchondral marrow of the vertebrae. The normal arrangement of the unaffected intervertebral disc and the epiphyses below serves for comparison. H. & E. stain. × 30.

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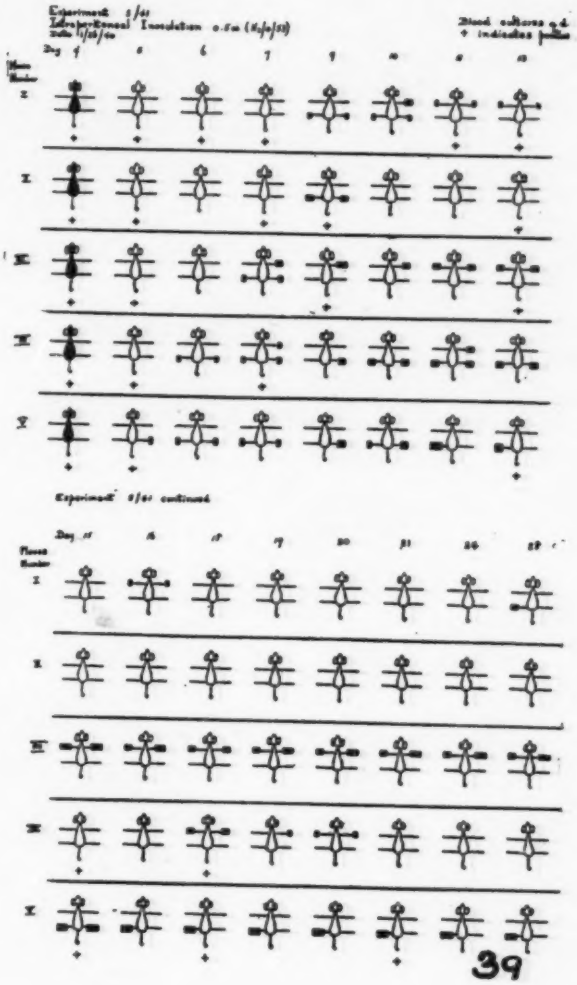
PLATE X

FIG. 39. Diagrammatic representation of the course of arthritis in mice following injection of the *Streptobacillus moniliformis*. The inked-in figures in the upper left-hand corner represent five 15 to 20 gram CFI strain mice, each inoculated intraperitoneally with 0.5 cc. of *Streptobacillus moniliformis* culture. The microorganism was grown in 6.0 cubic centimeters of 33 per cent ascitic fluid beef infusion broth; incubation 24 hours, 37°C. The 53rd generation of the streptobacillus strain from Case 1 was used. The streptobacillus had not undergone previous animal passage. The figures, arranged in parallel rows from left to right and continued in the lower section, represent repeated observations on each mouse at different time intervals following inoculation. The regions affected and the degree of arthritis are represented by the blocked in areas on the lines indicating the extremities. Blood cultures were taken on each day of observation. The plus signs indicate the positive blood cultures. Note the early migratory character of the arthritis, and the later tendency for the arthritis to become localized in one or more joints. The blood cultures were most frequently positive during the first two weeks following inoculation. The frequency of positive cultures gradually diminished after the 14th day and became uniformly negative after the 21st day.



PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in the Hurd Memorial Hall, January 12, 1942

The President of the Society, Dr. Frank B. Walsh, presided. Abstracts and discussions of the papers follow:

A Simple Method for the Laboratory Diagnosis of Subclinical Deficiencies of Thiamin, Riboflavin and Nicotinic Acid. L. Emmett Holt, Jr., and Victor A. Najjar (Department of Pediatrics).

In establishing laboratory criteria of these deficiencies the excretion of vitamins and certain vitamin derivatives in the urine has been utilized. The stores of thiamin and riboflavin are known to influence the excretion of these vitamins in the urine and we have shown that the urinary excretion of the fluorescent substance F_2 is dependent on available nicotinic acid.

In following the excretion of these factors several procedures have been employed: (1) *the 24-hour urinary excretion* is unsatisfactory, for it is influenced by the immediate diet regardless of the presence or absence of deficiency. (2) *An oral "load" test* in which excretion is followed for a period following oral administration of vitamin is not satisfactory because of variations in absorption which are encountered, particularly in gastrointestinal disorders. (3) *A load test with parenterally administered vitamin* is useful but subject to certain errors. A large, amount of vitamin is presented for excretion in a short time and renal insufficiency, when present, may modify the result of the test. The presence of deficiencies of other members of the B group also introduces complications. Further objections are the annoyance of the injection, the dislocation of meal schedules and the fact that only one deficiency can be tested at a time. We have recently employed (4) *a "morning hour" excretion test* to obviate these difficulties. Urine is collected for a period representing one hour's excretion following an overnight fast of 12 hours. This specimen is analyzed for thiamin, roboflavin and F_2 and the value so obtained appears to depend upon the body stores of thiamin, roboflavin and nicotinic acid. When the excretion of these factors in the one-hour specimen falls to zero it seems likely that the borderline of deficiency has been reached and that the body no longer has a surplus of vitamin available for excretion. Under these conditions it would seem desirable to supplement the diet even though clinical symptoms have not yet made their appearance. The morning hour excretion test will establish the presence of a degree of deficiency that needs dietary supplementation. It will not permit one to differentiate a mild deficiency which is just sufficient to produce a zero morning hour excretion from more severe degrees of depletion. To evaluate the more severe degrees of depletion by means of the laboratory, parenteral blood tests are still the method of choice.

DISCUSSION

Dr. Alfred E. Maumenee, Jr. said that a few cases of these vitamin deficiencies had been observed in the Wilmer Institute during the past year, some of them having been referred by Drs. Wintrobe and Miller because of ocular lesions thought to be due to riboflavin deficiency. Dr. Maumenee said that he had not found the typical vascularization of the cornea in these patients, but had observed a few cases of superficial corneal vascularization unaccompanied by mouth lesions. Two of these patients gave positive histories for riboflavin deficiency. In one case acne rosacea was present and the excretion test for riboflavin deficiency was positive; riboflavin was administered, and the symptoms cleared up within a week. The riboflavin excretion test on the second patient was normal, and the administration of riboflavin failed to clear up the ocular lesion.

Dr. W. Halsey Barker observed that the proposed test for determining nicotinic acid deficiency appeared to be superior to any of the methods previously used, such as tolerance tests. He said that many patients with fissures at the angles of the mouth showed no other evidence suggestive of riboflavin deficiency. The majority of patients with such mouth lesions whom Dr. Barker had seen were elderly women with false teeth, in whom the lesions may actually have been attributable to the constant moistening of the skin at the corners of the mouth with saliva during the night.

Carcinogenesis as a Conditioned Deficiency. CORNELIUS P. RHOADS. (Memorial Hospital, New York City.)

By a combination of chemical fractionation and qualitative spectroscopic methods the fate of dibenzanthracene was investigated in the excreta and certain tissues of mice, rats, rabbits, dogs, and monkeys. The spectroscopic investigations gave evidence for the presence of phenolic derivatives of dibenzanthracene in the excreta of mice, rats, rabbits, and dogs. No metabolite of dibenzanthracene was suggested in the excreta of monkeys. The spectroscopic investigation suggested that the phenolic derivatives of dibenzanthracene are identical in mice and rats and differed from the phenolic metabolite excreted by rabbits. No evidence was obtained for the excretion of a conjugated derivative of dibenzanthracene in any of the species studied. Small amounts of dibenzanthracene are excreted in the urine and feces of mice, rats, and rabbits. A preliminary quantitative estimation of the comparative ability of different species to convert dibenzanthracene shows that mice and rats convert only a small amount of the injected dibenzanthracene to the phenolic derivative as compared to rabbits. The phenolic derivatives of dibenzanthracene excreted by mice, rats, and rabbits were isolated. The phenolic metabolites of mice and rats are identical with 4′,8′-dihydroxydibenzanthracene synthesized by Fieser and Cason. The dihydroxydibenzanthracene produced by rabbits did not show carcinogenic activity when injected into mice. The chemical and biological significance of the conversion of dibenzanthracene to phenolic derivatives is discussed.

A method of preparing a stable apozymase is described. One metabolite (*p*-phenylenediamine) and a probable intermediary metabolite (N,N-dimethyl-*p*-phenylenediamine) of N,N-dimethylaminoazobenzene, which produces hepatic cancer in rats and causes a decrease of the diphosphopyridine nucleotide content of the damaged livers, have been shown to be toxic in low concentrations to a fermenting system in which diphosphopyridine nucleotide is the limiting factor. From a study of the metabolites and compounds related to them, it is concluded that the toxic effect is due to the formation of an intermediary oxidation product of the compound in the fermenting system. The acetyl derivatives of N,N-dimethyl-*p*-phenylenediamine and *p*-phenylenediamine are not toxic to the system. Alloxan has also been found to be highly toxic to the system. These experiments indicate that the inhibition of this system both by the Wurster type compounds and alloxan is a competitive one in which the substance competes with diphosphopyridine nucleotide for an enzyme active in fermentation.

DISCUSSION

Dr. Arnold R. Rich asked if the feeding of paraphenylenediamine alone produced carcinoma.

Dr. Rhoads replied that it did not. He said that both the paraphenylenediamine and the di-methyl-paraphenylenediamine were very labile and easily oxidized, the final oxidation products being inactive. Di-methyl-paraphenylenediamine is extremely toxic. He said that factors in the oxidation of phospholipids may also possibly be involved.

Dr. George O. Gey asked whether any of the substances studied by Chambers produced changes in normal liver cells in tissue culture. He was not aware that normal liver cells in culture had ever been changed to tumor cells.

Dr. Gey also inquired whether embryonic brei behaved like normal or like carcinomatous liver cells, and asked how regenerating liver cells behaved.

Dr. Rhoads replied that tumor cells had not developed from normal liver cells in tissue culture, possibly because the experiments had been very brief, designed only to show whether the cells survived or were killed.

Dr. Rhoads said that embryonic brei had behaved like the normal liver cells and the regenerating liver like the tumor cells in these studies, although the behavior was not identical. He observed that the livers in these feeding experiments were exposed to a constant toxemia, and that the tumor cells had developed a mechanism for avoiding this effect.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Synopsis of Genitourinary Diseases. By AUSTIN I. DODSON. 3rd ed. 302 pp. Illus. $3.50. *C. V. Mosby Company, St. Louis, 1941.*

Dr. Dodson, professor of genitourinary surgery at the Medical College of Virginia, has presented a comprehensive survey of factual urology in his 302 page book. The clinical material is concisely presented and is a valuable contribution for medical students.

With the exception of some good photographs, the illustrations are schematic drawings by Helen Lorraine that adequately depict the author's ideas. There are no reproductions of x-ray films; the urographic findings are illustrated by schematic drawings which are of little assistance to the student when he is confronted with actual films. It is unfortunate that authors are so often limited in the matter of illustrations by the efforts of the publishers to keep the price low.

In this latest edition of his book, Dr. Dodson has added some valuable dietary charts and has described the use of the various sulfonamide drugs in the treatment of urinary tract infections. The most gratifying addition to the third edition is the splendid presentation of the so-called functional disturbances associated with impotence and sterility.

L. G. L.

The Value of Health to a City. Two Lectures Delivered in 1873. By MAX VON PETTENKOFER. Translated from the German, with an Introduction by HENRY E. SIGERIST. 52 pp. $1.00. *The Johns Hopkins Press, Baltimore, 1941.*

The name of Max von Pettenkofer in this country is usually associated in an uncomplimentary way with his controversy with Koch and others over the importance of ground water in the causation of cholera. Lost is appreciation for his real contributions. He was a pioneer in hygiene and public health. In his time, such health regulations as were in force had depended upon empiricism. Pettenkofer was one of the first who sought to ascertain by scientific methods the conditions which were requisite for the optimum well-being of man. To this end he industriously collected information, performed experiments and disseminated knowledge. Following his appointment, in 1865, as the first Professor of Hygiene of the University of Munich, his interest was not confined to the sacred precincts of classroom and laboratory. The two lectures which are here presented were intended to "sell" to the citizens of Munich the desirability of betterment of the sanitary conditions and, in particular, the value of a good water supply to the city. Curiously enough, this German scientist paid high tribute

to advance already made in this direction by certain English cities. He proposed that Munich be made as healthy a city as London. It was in no small measure due to his interest and influence that this objective was subsequently realized. The example of Munich spread sanitary reform to other German cities. Thanks and appreciation are due to Dr. Sigerist for the translation and for his elucidation of the contribution which Pettenkofer made to the scientific, educational and practical advancement of hygiene and public health in Germany.

K. F. M.